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## Analysis of luminosity and redshift dependence of the large-scale distribution of quasars within the *Sloan Digital Sky Survey*

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### ABSTRACT

Quasars are the most luminous objects in the universe, emitting 100 times more energy than the entire Milky Way galaxy. Because their luminosities make them easy to observe at immense distances, quasars are useful when studying the large-scale structure of the universe. To better understand this structure, we have chosen to study the Sloan Digital Sky Survey Quasar Catalog. This dataset contains over 100,000 quasars and includes properties such as position, redshift (which correlates to distance), and luminosity. Using the pointwise dimension technique, which originates in the mathematics of fractal geometry, we analyze the environments of these quasars on numerous scales. We find two relationships between quasar properties and evolution of structure. We first compare the environments of quasars of different luminosities to each other. Most of the differences in those environments are found in the redshift range  $1 < z < 2$ . For each luminosity range, we then examine how specific environments around quasars evolved as a function of time. We find that the environments of bright quasars evolved in the distant past, while the environments of faint quasars evolved much more recently.

### INTRODUCTION

A galaxy is a gravitationally bound collection of stars, planets, gas and dust, surrounding a central supermassive black hole. Groups of galaxies can be gravitationally bound to one another within a galaxy cluster, which, in turn, can bind with other clusters to form enormous galaxy superclusters. The average density of a galaxy is about  $10^5$  times that of the universe as a whole. The average density of a galaxy cluster is  $10^2$  times that of the universe (Peebles 1993). These dense structures indicate that the universe is heterogeneous; matter is clumped together and not evenly distributed. However, a different picture of the universe emerged with the 1965 discovery of the Cosmic Microwave Background (CMB).

The CMB was discovered by Bell Laboratories scientists Arno Penzias and Robert Wilson. While they were conducting experiments on a very sensitive antenna for satellite devices, they observed a constant level of background noise in every direction that could not be accounted for by instrumental noise or atmospheric emission (Murdin 2001). Subsequent research measured the intensity of

this background noise at different wavelengths. After correcting for errors attributed to the motion of our solar system, any further anisotropy (irregularity in the distribution) fell beneath the sensitivity of the instruments being used (Murdin 2001). This background noise is the same in every direction so it is defined as isotropic. This signal, now called the CMB, is the signal that remains from the time of recombination in the very early universe. This radiation essentially gives a snapshot of how the early universe looked. The CMB seems to be showing that the universe is, on the largest scales, smooth, or the same in all directions, which supports an idea known as the Cosmological Principle.

The Cosmological Principle states that at the largest scales the universe is isotropic (the same in every direction), and homogeneous (the same density in all locations). Because the CMB shows that the universe is isotropic on the largest scales, it is primary evidence that supports the Cosmological Principle (e.g., Wu *et al.* 1999). A contemporary problem in cosmology is to find the scale at which the universe transitions from local clustering in

the dense galaxies and galaxy clusters to the isotropic and homogeneous structure that the CMB indicates.

Experiments continued into the 1990s to try to reconcile the homogeneous view of the universe from the CMB with the non-homogeneous large-scale structure of the universe observed in the clustering of matter. To this end, the Cosmic Background Explorer (COBE) was launched in 1989 to search for spectral distortions and anisotropy (as described in Coles 2005). In 1990, it was announced that the CMB spectrum measured by COBE showed no deviations from a blackbody radiation distribution, meaning that the universe is an isothermal object (an object at a constant temperature) that absorbs all incident radiation and it is a perfect emitter (Murdin 2001). The Wilkinson Microwave Anisotropy Probe (WMAP) is a more recent experiment launched in 2001 to map temperature fluctuations in the CMB. Evidence from WMAP that the universe is isothermal at the largest scales supports the Cosmological Principle that the overall distribution of matter in the universe (on large scales) is smooth.

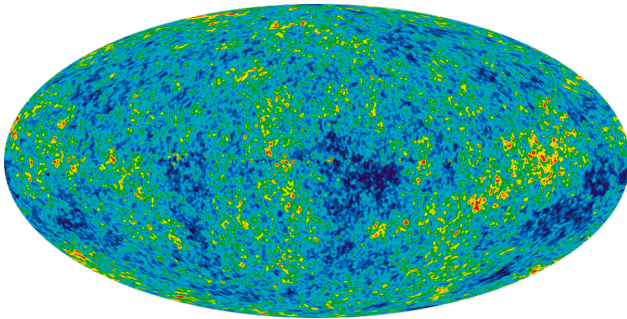


Figure 1: Full sky image generated by the Wilkinson Microwave Anisotropy Probe. Courtesy NASA.

Although the CMB is isotropic overall, small anisotropies, or irregularities, can be found by magnifying the signal. Figure 1 shows the irregularities in the temperature of the universe. These fluctuations are very small, on the order of 0.0002 Kelvin. Although tiny, the locations

of these anisotropies could possibly have been “seeds” for areas of higher density in the universe. By comparing those “seeds” to the locations of galaxies and galaxy clusters that create the large-scale structure of the universe, we may be able to reconcile the isotropic and homogeneous early universe with the irregular, clumped universe of the present (Coles 2005).

To better understand the large-scale distribution of galaxies, surveys of as much of the sky as possible are critical. These surveys, called “all-sky surveys”, provide a broad picture of the distribution of astronomical objects in the universe. The Palomar Observatory Sky Survey (POSS), conducted between 1949 and 1958, was one of the first all-sky surveys. It collected the angular positions and luminosities of celestial objects in approximately 2/3 of the entire sky (Djorgovski 2012). All-sky surveys such as POSS are the precursors of many other catalogs of celestial objects, such as galaxies and quasars, that help trace the large-scale structure of the universe. Recent surveys include the Las Campanas Redshift Survey (Shectman *et al.* 1996), the Sloan Digital Sky Survey (Abazajian *et al.* 2003), the DEEP2 Redshift Survey (Davis *et al.* 2003), and the 2 Micron All Sky Survey (Skrutskie *et al.* 2006). These and other catalogs of galaxies generated by surveys of the large-scale structure of the universe are being used to produce a picture of the current state of the universe, as statistical analyses can be performed on these catalogs to produce a description of the clustering phenomena in the universe. We are interested in the clustering patterns and any correlated factors that may contribute to the evolution of structure in the universe. By observing astronomical objects (such as galaxies) on numerous distance scales, we can observe the environments of those objects at specific epochs and construct a history of structure evolution. This is challenging because galaxies at such a large distance are difficult to observe, but by using a bright tracer object, such as a quasar, the difficulty is decreased (West 2003).



Quasars are the brightest objects in the universe. Quasars occur when a supermassive black hole (SMBH) at the center of a galaxy is fed by material that falls into an accretion disk surrounding the SMBH, as shown in Figure 2.

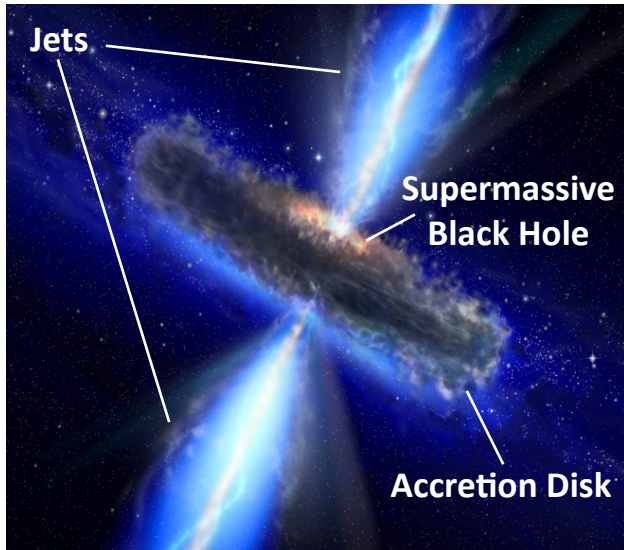


Figure 2: Artist's rendition of the structure of a quasar. Courtesy NASA.

When fed by this material, the SMBH can eject a jet of material at very high speeds that emits over all wavelengths of the electromagnetic spectrum. In most galaxies today, the SMBH is starved of interstellar medium and does not eject a super-emitting jet, but in galaxies at higher redshifts (corresponding to earlier times in the universe) many more of these emitting black holes exist. In some cases, the jets of material from the black hole far outshine the light from the surrounding stars in the galaxy, and are the brightest objects in the universe. The brightness, or luminosities, of quasars makes them of particular interest to the study of structure evolution. In this study, we use the Sloan Digital Sky Survey Quasar Catalog Data Release 7 (Schneider *et al.* 2010) to compare the environments of quasars based on various properties.

## METHODS

Structures in nature, full of complex shapes and patterns, are not always easily described with traditional mathematical tools. In these

cases, we must therefore turn to nontraditional tools. A fractal is an irregular shape that cannot be described by regular geometric shapes, and is self-similar: that is, a fractal looks (approximately) the same at any scale (as described in Falconer 2003). Another important characteristic of a fractal is that its dimensionality cannot typically be described using whole numbers.

Fractals can be used to describe many systems that occur in nature. Fractals can be used in earth science to describe rivers, in biology to model blood vessels, in chemistry to describe the patterns of polymers, and in physics to describe the surfaces of solids. Each of these systems exhibit self-similarity and they all may occur on many different scales.

Four decades of observations of galactic clustering show approximate self-similarity on numerous scales. Fractals are particularly useful for the study of galaxy and quasar distributions (e.g., Yadav *et al.* 2010). Quasars are grouped in highly structured hierarchical patterns that exhibit properties of self-similarity, as shown in Coleman and Pietronero (1992) and Bottoroff and Ferland (2001). As such, fractal mathematics can be applied to quantify the environment surrounding quasars in a large sample survey.

In this work, we will quantify the environments of quasars and attempt to determine if there is any relationship between luminosity, redshift, and structure evolution using the pointwise dimension (as defined in Mayer-Kress 1994 and applied in Best, Charlton and Mayer-Kress 1996).

The pointwise dimension considers the function  $N_{x_m}(r)$ , which is the count of the number of data points  $N$  within distance  $r$  from a reference point  $x_m$ . The results of this calculation can be plotted in a log-log representation (as seen in Figure 3) over which there is a scaling region bound by  $r_{\min}$  and  $r_{\max}$ ; the slope  $d_{x_m}$  is interpreted as the pointwise dimension.

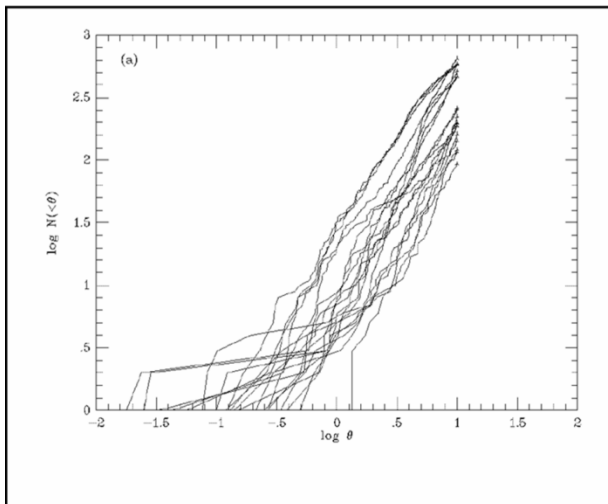


Figure 3: Example plot of the environment curves (one per astronomical object) used to generate the pointwise dimension. Courtesy J. Best.

The dimension is a measure of the environment around the quasar, and may be analyzed on various scales (known as fitting ranges) by limiting the region over which the slope is fitted (Best, Charlton and Mayer-Kress 1996).

The Sloan Digital Sky Survey (SDSS) is the most comprehensive observational survey to date. In the eight years of operation from 2000-2008, the survey created a map of the universe encompassing more than 8,400 square degrees of the sky and obtained spectra of more than 930,000 galaxies, 120,000 quasars, and 225,000 stars (Schneider *et al.* 2010). The spectroscopic data collected from SDSS Quasar Catalog Data Release 7 includes physical properties of quasars such as positions (right ascension [RA, or  $\alpha$ ] and declination [DEC, or  $\delta$ ]), magnitudes (which corresponds to luminosities), and redshifts (which corresponds to distances) as detailed in Schneider *et al.* 2010. These data are tabulated and released to the public in small increments. The sample size we analyzed has been limited to two small sections in order to eliminate data edge effects. The larger section of data has been limited to include only those quasars within an RA range of  $140^\circ < \alpha < 200^\circ$  and DEC range of  $0^\circ < \delta < 60^\circ$ , while the

smaller region spans an RA range of  $320^\circ < \alpha < 30^\circ$  and DEC range of  $-10^\circ < \delta < -5^\circ$ . The regions were then combined for data analysis.

## RESULTS AND ANALYSES

A Kolmogorov-Smirnov test (KS test) is used to compare the environments around each quasar. A KS test is particularly useful in statistics when the distribution of data is non-parametric. Since the distribution of matter in the universe is not known, using the KS test reduces any error that would occur based on an inaccurate model of data distribution. In this study, we use the KS test to compare the environments around each quasar to find the probability that they are drawn from the same parent population. If we find that the probability that the two quasar environments are drawn from the same distribution to be less than 1%, we report the quasars as not statistically similar to the 99% confidence level, and denote that result with the letter “N”. If the quasar environments are not statistically similar, we say that evolution has occurred in the environments of those quasars. If the probability that the quasars are from the same parent population is above 1%, we denote that comparison with the letter “Y”. In that case, we cannot conclude that any evolution of the environment has occurred. We designed comparisons that examined the dependence of quasar environment evolution on redshift and luminosity criteria.

We first compared the environments of quasars of different magnitudes to each other at specific fitting ranges around each quasar, for all redshifts and within specific redshift slices. The results of those comparisons are shown in Tables 1a-1d. We then compared quasars at different redshifts to each other at specified fitting ranges, constrained by specific magnitude values. The results of these tests are in Tables 2a-2c.

For all tables: The magnitude scale in astronomy is a measure of the inherent brightness of an object, and is defined such that





## DISCUSSION AND CONCLUSIONS

In Table 1a, we compare quasars of different magnitudes over the entire redshift range of our data. An analysis of Table 1a shows that there is evolution in quasars of all magnitude comparisons, except for the brightest quasars in smaller environments. In Tables 1b and 1c, we compare quasars of different magnitudes to one another at different redshift slices. An analysis of Table 1b (nearby redshifts) shows that the fainter quasars have evolution of structure, whereas brighter quasars do not. Table 1c shows that at farther redshifts, all quasars except for the brightest magnitude comparisons show evolution of structure. In Table 1d, only the medium brightness quasars show evolution of structure. Overall, we see evolution in faint quasars and no evolution in bright quasars. This suggests that the evolution in the environments of the fainter quasars happens between redshifts  $z=1$  and  $z=3$ . These data show that when comparing quasars by luminosity, there is a redshift dependence to the evolution. Nearby, only the faintest quasars evolve; in medium redshift ranges, all but the brightest quasars evolve; at largest redshift ranges, only the medium range quasars evolve. While numerous studies (e.g., Croom 2002; Lidz 2007; Shen *et al.* 2012) find some relationship between luminosity and evolution, none describe as specific a relationship as we have found here. Our result also stands in contrast to White *et al.* (2012), who find no strong link between luminosity and evolution.

In Tables 2a and 2b, we compare quasars of specified magnitudes in different redshift ranges to each other. In Table 2a (closer redshifts), we find that fainter quasar environments evolve, while brighter quasar environments do not. Table 2b shows that at greater redshifts, the brighter quasar environments evolve while the fainter quasar environments show no evolution, even holding magnitude constant. In Table 2c, near and far redshifts are compared to each other and we find that only the intermediate luminosity ranges show quasar environment evolution. Therefore, we conclude that there is

a relationship between redshift and evolution of structure. Faint quasar environments evolved recently, and bright quasar environments evolved in the more distant past. This finding extends work by others (e.g., Bagla 1998; Porciani *et al.* 2004) as well as our own previous findings (Thompson and Best 2005; Hanni and Best 2008) which all find a relationship between redshift and evolution.

We studied the structure of quasar environments as a function of both redshift and magnitude to try to understand the factors that might contribute to how the universe transformed from the smooth CMB to the current clustered universe. Our discoveries about the relationship between luminosity and structure evolution could help achieve further understanding about the origins of the structure of the universe.

## REFERENCES

- Abazajian, K. *et al.* 2003. *AJ*, **126**: 2081-2086.
- Bagla, J.S. 1998. *Evolution of Galaxy and Quasar Clustering*. *MNRAS*. **297**: 251-254.
- Best, J. 1999. *Analyzing the Dressler Catalog using the Pointwise Dimension*. *APJ*. **520**: 507-513.
- Best, J. 2000. *An Examination of the Large-Scale Clustering of the Las Campanas Redshift Survey*. *APJ*. **541**: 519-526.
- Best, J., J. Charlton, and G. Mayer-Kress. 1996. *Analysis of Galaxy Morphology and Evolution using the Pointwise Dimension*. *APJ*. **456**: 55-64.
- Bottoroff, M. and G. Ferland. 2001. *Fractal Quasar Clouds*. *APJ*. **549(I)**: 118-132.
- Coles, P. 2005. *The State of the Universe*. *Nature*. **433**: 248-255.

- Coleman, P. and L. Pietronero. 1992. *The fractal structure of the universe*. Physics Reports. **213**(6): 311-389.
- Croom, S. *et al.* 2002. MNRAS. **33**: 459-464.
- Davis, M. *et al.* 2003. *Discoveries and Research Prospects from 6- to 10-Meter-Class Telescopes II*. Edited by Guhathakurta, Puragra. Proceedings of the SPIE. **4834**: 161- 172.
- Djorgovski, S.G., A. Mahabal, A. Drake, M. Graham, and C. Donalek. 2012. *Planets, Stars, and Stellar Systems*. Springer Verlag, in press.
- Hanni, R. and J. Best. 2008. *Analysis of Large-Scale Distribution of Quasars Within the Sloan Digital Sky Survey Quasar Catalog*. Proc. WVAS. **80**:1-8.
- Lidz, A. *et al.* 2006. APJ **641**: 41-49.
- Mandelbrot, B. 1983. *The Fractal Geometry of Nature*. W.H. Freeman. New York. 468 pp.
- Mayer-Kress, G. 1994. *Localized Measures for Non-Stationary Time-Series of Physiological Data*. Integrative Physiological and Behavioral Science. **29**(3): 203.
- Murdin, P. 2001. *Encyclopedia of Astronomy and Astrophysics*. Institute of Physics Publishing. 3670 pp.
- Peebles, P.J.E. 1980. *The Large-scale Structure of the Universe*. Princeton Univ. Press. Princeton. 440 pp.
- Porciani, C. *et al.* 2004. *Cosmic evolution of quasar clustering: implications for the host haloes*. MNRAS. **355**: 1010-1030.
- Schneider, D. *et al.* 2010. *The SDSS-DR7 Quasar Catalog*. APJ. **139**: 2360-2373.
- Shectman, S. A. *et al.* 1996. *The Las Campanas Redshift Survey*. APJ. **470**: 172-212.
- Shen, Y. *et al.* 2012. *Cross-Correlation of SDSS DR7 Quasars and DR10 BOSS Galaxies: The Weak Luminosity Dependence of Quasar Clustering at  $z \sim 0.5$* . APJ, submitted.
- Skrutskie, M. F. *et al.* 2006. *The Two Micron All Sky Survey*. AJ. **131**(2): 1163-1183.
- Smoot, G. 2007. *Nobel Lecture: Cosmic microwave background radiation anisotropies: Their discovery and utilization*. Reviews of Modern Physics **79**: 113-166.
- Thompson, J. and J. Best. 2005. *Analysis of Structure Evolution within the Sloan Digital Sky Survey Quasar Catalog*. Proc. WVAS. **77**: 14-22.
- West, M. *et al.* 2003. *Reconstructing Galaxy Histories from Globular Clusters*. Nature. **427**: 31-35.
- White, M. *et al.* 2012. *The clustering of intermediate-redshift quasars as measured by the Baryon Oscillation Spectroscopic Survey*. MNRAS. **424**: 933-950.
- Wu, K. *et al.* 1999. *The Large-Scale Smoothness of the Universe*. Nature. **397**: 225-230.
- Yadav, J. K., J. S. Bagla, and N. Khandai. 2010. *Fractal Dimension as a measure of the scale of homogeneity*. MNRAS. **405**(3): 2009-2015.

## The West Virginia contribution to America's first successful asphalt paving

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### ABSTRACT

Numerous stories relate that asphalt from Ritchie Mines in West Virginia was used to build early asphalt streets in America. The earliest such street was Fifth Avenue between 24th and 25th streets on Manhattan. It was started in 1872 and completed in 1873 by the Grahamite Asphalt Paving Company. In 1870, de Smedt was awarded a U.S. patent for mixing sand, gravel, asphalt and heating the mixture. Just before it was laid down, a small amount of "Ritchie mineral", scientific name grahamite, was added to allow the pavement to withstand the summer heat. Perhaps the most interesting aspect of this project is the modification of the cheaper Trinidad Lake Asphalt with grahamite to yield a satisfactory pavement. This project was the first example of an asphalt pavement in the United States. This paper documents the use of West Virginia grahamite in the earliest asphalt pavement in the U.S.; a contribution that has been largely ignored by the asphalt industry's perception of its history.

### BACKGROUND

Asphalt mining in Ritchie County, West Virginia in the mid 1800s is well documented (Grimsley 1910; Lowther 1911; Pepper 1939; Hodge 1992; Ritchie County Tourism and Visitor's Bureau, Inc. 2009; Murphy 2008; and Bartlett 2010). Most of the accounts emphasize the narrow gauge railway that took the asphalt to market, or the explosions in the mines, or the boom town aspect of the mining. In a few cases, however, mention is made that this asphalt was used to pave the first successful road in America in 1872. Is there truth to this assertion, or is it merely embellished local history?

### THE STATE OF THE ART IN ROAD BUILDING IN 1870

In the 1870s, the options for transportation were limited to waterways, railways, and roads. Road is defined here as a public way for the passage of vehicles, people, and animals. In the period of interest, the vehicles were carts and wagons pulled by people, horses or other draft animals. By 1870, there were American

textbooks on road building (for example, Gillespie 1849). Road designers were primarily interested in the road geometry, surface, and the pavement structure.

The considerations for paving streets and roads in 1870 centered on horses or horse-drawn wagons and carts. Key issues included:

- traction was generally by horses or horse-drawn conveyances,
- the resistance of drawn vehicles over the road surfaces to the horses' efforts to pull,
- roads required considerable cleaning because of the horses,
- the pavements could not be so slippery that the horses fell, and
- some surfaces were noisy with horses and horse drawn transport.

The Industrial Revolution generated the need for roads for the movement of goods.

Early attempts to improve roads used a variety of materials such as cobblestones, Belgian blocks, granite setts, flagstone and even wood. The most successful pavements were constructed with broken stone. The first important advance in broken stone pavements was made by a French engineer, Pierre-Marie-Jérôme Trésaguet, in the mid-1700s. However, the French pavements had drainage problems that kept the roads from being completely satisfactory. In the late 1700s, two British engineers, Thomas Telford and John Loudon McAdam, improved on Trésaguet's design by elevating the pavement structure to permit drainage. Telford's roads used large, carefully selected and placed stones as a foundation in the base layer. McAdam had the insight that relatively small, angular stones would carry the traffic load satisfactorily. Telford's design lasted longer, but McAdam's design was more economical and eventually predominated. The Macadam and Telford pavement structures provided adequate structural capacity for the traffic of the day, but the lightly-bound broken-stone surfaces were not ideal for horses, carts, and wagons (and even less so with the advent of the automobile).

The idea for paving roads using asphalt seems to have come from the area around Val-de-Travers, Switzerland, where nearby roads were haphazardly paved when asphalt-bearing rocks that fell off the carts were ground under the cart wheels. An experimental road was constructed in France, between Bordeaux and Rouen, by using a structure based on McAdam's, and then covering the surface with an inch and a half of small broken rocks from the asphaltic limestone quarries. Although initially successful, granite from the macadam course eventually worked its way up through the asphalt layer. A Swiss engineer, A. Merian, had the idea of applying warmed, powdered asphalt-bearing rock to the surface and compressing it. This idea was used on the Rue Bergere in Paris in 1854 (anonymous 1873; Law and Clark 1877). Work in London between May 1869 and 1873 resulted in 60,800 square yards of both compressed and mastic (poured) asphalt.

Prior to 1870, asphalt paving was essentially non-existent in America. de Smedt, a Belgian chemist, studied asphalt paving in Paris. He immigrated to the United States in 1861 and worked on coal dust at Columbia University (de Smedt 1893). In 1869, his interests turned to asphalt paving. There were trials of imported Val-de-Travers material which were not completely satisfactory. Apparently, about this time he had the insight that point to point contact in coarse stone bound by asphalt would offer an advantage by providing a stiff layer. This would resist the pressure from the traffic passing over the layer. Since the wheels of that time had narrow metal rims and not pneumatic rubber tires, the pressures exerted by passing wagons were substantial. In 1870, de Smedt was granted two patents for asphalt paving.

#### **THE FIRST SUCCESSFUL ASPHALT PAVING IN AMERICA**

Contemporary asphalt paving practice is described in Gillmore (1876). Natural asphalt rock used in Europe at the time was a weak asphalt-impregnated limestone which would crumble into a powder upon heating between 200 to 212 °F. The hot powder was then packed with heated tampers, rammed, or rolled to compress it (Gillmore 1876; North 1879).

In the summer of 1870, de Smedt laid down about 500 square yards of hot mix asphalt paving in front of the Newark, New Jersey, City Hall. The mix was probably what is called a sand mix, i.e. no aggregate larger than sand size. Sand mixes are no longer used.

Many people consider this to be the first asphalt pavement in America. In a sense, it was; however, it was only partly successful and is not representative of current asphalt paving practices. Photographs of old City Hall in Newark at the corner of William Street and Broad Street from the early 1900s (Old Newark 2013) show Belgian block indicating the asphalt placed in 1870 was removed, either intentionally or by wear. Technical opinion several years after 1870, after more pavements had been



laid, was that the Newark test patch had given “encouragement to perfect machinery and appliances for properly handling the materials”. Regarding the test patch itself, opinion was that: “For this work the crudest implements were used, and much of the material was burnt and otherwise injured in the process of mixing and laying.” (anonymous 1885). Despite these problems, de Smedt persevered. One of his patents, 103582, (de Smedt 1870) can be viewed as the precursor to today’s hot mix asphalt. De Smedt’s innovation was mixing aggregate with asphalt rather than looking for a natural rock with the desired properties. He specified that the aggregate and asphalt were to be heated, mixed and placed hot. Although not specified by the patent, the first de Smedt paving seems to have been hand-tamped.

**THE COMPONENTS OF THE DE SMEDT PATENT**

De Smedt’s patent gave both a composition of the asphalt paving and a method of applying the paving to the road.

Table 1. Composition of de Smedt’s patented asphalt

component	proportion (%)
sand or powdered stone	37.50
gravel or broken stone	55
asphalt	6.25
petroleum oil	1.25
albertite or Ritchie mineral	0.25 to 2.5

Sand is both a material and a size fraction. As a size fraction, de Smedt’s use of sand approximately serves the same purpose that fine aggregate serves in today’s hot mix asphalt. As a material, natural sand was available in the New York City area where de Smedt was working. Large amounts were taken from nearby glacial moraines in the succeeding decades. By powdered stone, de Smedt probably meant a crushed stone in the sand size range. Stone sand, or manufactured sand

as it has been called, existed in 1870 as a by-product of breaking stones, but its use was not widespread until the 20th century, particularly after World War II (Renninger and Nichols 1968; Gray and Bell 1964).

Both the gravel and the broken stone in de Smedt’s patent refer to what would now be called coarse aggregate in hot mix asphalt. Gravel is available in the New York City area from the glacial moraines. Like sand, large amounts of gravel were mined at Port Washington on Long Island in the decades following de Smedt’s patent. Gravel is usually at least somewhat rounded by transport and deposition. Since the time of McAdam it had been realized that the interaction of angular, broken stone was a major factor in the strength of a broken stone roadway. Originally, the stone was broken by hand using a stonebreaker’s hammer, but by de Smedt’s time the work was increasingly being mechanized. De Smedt would allow either, but called for enough coarse stone to develop an aggregate skeleton.

The source of asphalt most readily available on the Atlantic and Gulf Coast of North America was from the Pitch Lake on the island of Trinidad, currently called Trinidad Lake Asphalt, TLA. It had been known to Europeans since Sir Walter Raleigh described and used it in February 1595, and was the largest known asphalt deposit in the world during de Smedt’s time (Richardson 1905; Lake Asphalt of Trinidad and Tobago 2014). The TLA was easily mined, but it had some tendency to fuse in the holds of ships during transport, so that it had to be remined on arrival (Lucier 2008). There was some reluctance to carry it. At the time, rights to mine it were held by the British Admiral Cochrane, 10th Earl of Dundonald, who took out patents in the 1850s for various uses but not including paving. Commercial mining of the TLA started in 1864. Following de Smedt’s success, A.L. Barber gained a 21-year concession for the mining rights in 1888 (anonymous 2013). The Venezuelan Bermudez deposit, Trinidad Lake’s main competitor among

natural asphalts was not yet discovered; asphalts manufactured during crude oil distillation were not known yet.

Trinidad Lake Asphalt has a high viscosity and a very hard consistency, probably stemming from the natural clay content. The ability to heat the paving mix in the 1870s did not reduce the viscosity of the asphalt enough to allow it to adequately coat the aggregates. A “flux” blended with the TLA could produce a material with the needed flow for the mixing and construction process. The “petroleum oil” mentioned in the patent was probably what is now called kerosene. Recently invented, at that time kerosene was made from albertite, cannel coal (hence the alternate name of coal oil), or crude oil after the Drake well in northwestern Pennsylvania in 1859 brought the modern petroleum (oil) industry into existence. However, the mix of the TLA and kerosene would not have sufficient stiffness to carry traffic immediately following construction. It appears that de Smedt solved this problem with the addition of a very stiff asphaltic material following the mixing of the other components. Two suitable materials were available at the time, albertite and grahamite.

Albertite is a black earth material initially described by Gesner, named after Albert County during the first geological survey of New Brunswick, Canada, in the late 1830s (Lucier 2008). A long court case based on British mineral law hampered exploitation. It was incorrectly decided in court that it was coal (Lucier 2008). It is now thought to be a natural derivative of oil shale (Dyini 2005). The main mining was done from 1863 to 1874. In the United States at this time, 140,000 tons were sold at a price of \$18/ton. Baskerville (1909) gives a production figure of 154,800 tons for this era with an estimated total production of over 200,000 tons. He gives the value at between \$15 and \$20/ton. Macfarlane (1875) reports albertite production in 1869, 1870 and 1871 to be 19,267 tons.

## GRAHAMITE OR RITCHIE MINERAL

Grimsley (1910), citing Miss Lowther of Smithville, West Virginia, gives the history of the Ritchie County asphalt that was published in the Harrisville *Ritchie Standard*. It was discovered by Lemon in 1852, who thought it was coal and hid it until he could buy the property. Exposed again by erosion in 1858, people started using it, especially for blacksmiths’ forges. In that year, Lemon bought the property from John Webb and Robert Marshall, but sold the property the next year to Beall of Frostburg, Maryland, who started mining the material, but unsettled conditions during the Civil War led to a stoppage of the work. During or shortly after 1865, Beall sold the property to a syndicate from New York and Baltimore. The Graham brothers were the principal members of the syndicate which built a narrow gauge railway nicknamed the Calico Railroad from the property to the main B&O railroad line. Reportedly, during mining in the 1870s, 15 to 20 rail cars a day were shipped, and over \$100,000 was spent on equipment, although it is unclear if this was only on the railroad or included the mine itself.

The first mention of the deposit in the scientific literature was Lesley (1863), who communicated to the American Philosophical Society information given him in a letter by Gratz of Philadelphia. Although Lesley seems to have understood the geology reasonably well, it appears that he had not visited the site himself. He mentions mining and experimentation both in the laboratory and on the site concerning the yield of liquid from the material. No attempt to name the material was made other than calling it both coal and asphalt. Professor Wurtz coined the name grahamite in deference to the principal owners of the mineral (Lucier 2008; anonymous 1865). Wurtz identified possible uses such as illuminating oils and gases, bottle sealing cement, translucent varnishes and lubricating compositions, but apparently did not consider in asphalt paving.

A description of the geology of the grahamite deposit was made in 1873 by Professor Fontaine

of West Virginia University (Fontaine 1873) after a visit to the site accompanied by William Glenn, a civil engineer who was the superintendent of the mines and railway for the Ritchie Company. Fontaine clearly describes grahamite as an asphalt, and notes that it was used in the production of gas, especially to enrich the coal that was the other material used in the gas production. He noted the similarity to albertite. The grahamite filled a vertical fissure through Pennsylvanian age sedimentary rocks. Where the grahamite was bounded by sandstone it reached a maximum width of four feet but tapered to a few inches at either end horizontally. Where the wall rock was shale the grahamite was only two and a half feet wide. The descriptions which survive are too vague to accurately estimate a recoverable tonnage-in-place, but the resource was probably not large. Fontaine remarks that in place the grahamite looks similar to bituminous coal, which probably explains why it was thought originally to be coal. The portion of the deposit next to the wall rock was described as having a brilliant luster, jet-black color and breaking into short prisms. The inner portion is described as having dull to resinous luster and a dark steel gray color. The grahamite was very friable and produced a fine powder during handling.

An account of the mining itself was given by Glenn (Glenn 1895). The focus of Glenn's paper was the explosions at the mine in 1871 and 1873. Much of what was written was reconstructed from memory, but he was able to draw maps of the mine at the time of the two explosions and to describe the methods of mining. Mining was from below using a light coal pick. The finely broken material was then loaded out and transported by rail. Mining seems to have stopped soon after the second explosion. Perhaps the explosions caused the shut-down; perhaps unfavorable market conditions were responsible, or perhaps some combination. Mining was briefly resumed in the early 20th century, so complete resource exhaustion was probably not the cause. The Ritchie County deposit was relatively small,

even compared to the Albert Mines. Macfarlane (1875) gives the amount of grahamite shipped in 1870 as 1,174 tons and remarks that the price in New York was a little less than the price of albertite.

### **THE FIFTH AVENUE PAVEMENT OF 1872-1873**

Pennsylvania Avenue in Washington, D.C., in 1876 is often considered to be the first asphalt paved street or road in the United States. However, a report by van Nort, Commissioner of Public Works, to the Board of Aldermen of the City of New York dated October 15, 1874 (anonymous 1877) clearly describes the paving of 5th Avenue between 24th and 25th Streets using de Smedt's patent. In the fall of 1872, a fifteen foot test strip was laid down, and in the spring of 1873 the entire block was paved. The foundation was Belgian block laid in a sand base which was lowered four inches and then overlaid with "Grahamite Asphalt Pavement" by the Grahamite Asphalt Company. This was done as a demonstration project at no cost to the city, since patented pavements were illegal in New York City at the time. Although the de Smedt patent allowed either grahamite or albertite, grahamite was probably used, since the company owned the grahamite mine and because contemporary accounts (North 1879; Gillmore 1876) mention grahamite as being used.

The block of 5th Avenue opposite the Worth Monument was the severest test of the de Smedt patent and of the Grahamite Asphalt Company pavements, due to both high traffic count and heavy vehicles, but there were other jobs at this time. In the summer of 1872, the pavement was used as an overlay ("blacktop") for an old tar pavement on a block of 38th Street. Similarly, in the summer of 1873, a block of 18th Street was repaired. A block of 28th Street was repaired in the fall of 1873. A block of Thomas Street was paved with the mix put over hydraulic concrete and paid for by the adjacent property owners. The 28th Street job failed due to poor workmanship,

but the other jobs were considered successful. There are testimonials to the success of the 5th Avenue job from George Greene and Isaac Newton, civil engineers Charles K. Graham (no relation), Engineer-in-Chief of the Department of Docks, and Lt. Col. H.G. Wright of the U.S. Army Corps of Engineers (anonymous 1875; North 1879; Gillmore 1876). The success led to de Smedt moving to Washington, D.C. in 1876 after President Ulysses S. Grant called for making Washington “a city worthy of the nation’s capital (Poore 1872). While the paving of Pennsylvania Avenue in the District of Columbia is considered the first “pavement on an extended scale” (anonymous 1885), it was successful pilot projects in the previous few years that allowed the trial of a full-scale project on Pennsylvania Avenue. Contemporary opinion (anonymous 1885) was that the pilot projects (“little more than good-sized samples”), notably the Fifth Avenue project, which were laid with “somewhat varying success, though no positive failure” led to the trial on Pennsylvania Avenue, Washington. “It was these specimens that first fully demonstrated the durability of the pavement under the severest tests of heavy traffic and changing temperature that those cities afford.” (anonymous 1885).

The diffusion of knowledge about the new pavement was rapid. In 1868, a leading American encyclopedia’s article on pavement makes no mention of asphalt paving (Ripley and Dana 1868). By 1875, the article had been revised to mention the new pavement type and the contribution of grahamite from West Virginia (Ripley and Dana 1875).

### **THE SIGNIFICANCE OF GRAHAMITE, THE DE SMEDT PATENT AND THE FIFTH AVENUE PAVING JOB**

Native asphalt trials had been conducted unsuccessfully by de Smedt in Newark, N.J. in 1870, and by others in this time period in Philadelphia, New York, Washington and Chicago. Manufactured asphalt blocks, stone blocks, wooden blocks, and paving bricks were also being considered as paving materials.

Cement already had some uses and Portland cement concrete designs would compete with asphalt in the future.

Asphaltic concrete has become the predominant road paving method, but was the de Smedt demonstration paving project on Fifth Avenue successful or merely a very early example of asphaltic concrete paving? Holley (2003) in his detailed history of the beginning of asphalt paving in the United States references an 1869 letter to the editor of the *New York Times* (anonymous 1869) about problems with asphalt paving of Fifth Avenue opposite the Stewart mansion, at the corner of Fifth Avenue and 34th Street, deteriorating in three weeks and asserts that the asphalt paving was a disaster. This could not have been the de Smedt demonstration project for two reasons; first, the *New York Times* article was dated October 9, 1869 preceding construction of the de Smedt project in 1872-1873 and secondly, the Stewart mansion is ten blocks from 24th and 25th Streets, where the de Smedt pavement was constructed.

The de Smedt project is remembered as being successful. One contemporary account is in a detective story from 1881 where the hero is taken to a mysterious rendezvous in a closed carriage. Being able to figure out where he was taken hinges on passing over the smooth block of Fifth Avenue opposite the Worth Monument (Green 1881). The author, an early woman writer of detective stories, would have been unlikely to include this detail if it had not been true and familiar to at least some of the intended readers.

The reaction of the technical community concerning the success of the de Smedt project is reflected in Commissioner van Nort’s report, which was reprinted as part of the public campaign to use such paving elsewhere on Fifth Avenue (anonymous 1875). That block of Fifth Avenue was much in the public mind.

The successful asphalt paving of Fifth Avenue was long remembered. In the U.S. Senate hearing by the Subcommittee of

the Committee on Appropriations for the appropriations bill for the District of Columbia government for the year ending June 30, 1907, Senator Wetmore from Rhode Island in the afternoon of May 26, 1906 questioned Colonel Biddle, Engineer Commissioner for the District, about the need for repaving. Colonel Biddle stated that some streets in New York City with heavy traffic required repaving in 10 years. Senator Wetmore remarked that there is good pavement opposite the Worth Monument which he supposed had lasted 30 years. Colonel Biddle replied that some District of Columbia pavements were in good shape after 20 years. Senator Wetmore's rejoinder was that the traffic in front of the Worth Monument was, of course, enormous (anonymous 1906).

Wood (2012) points out that civil engineers need to economically and efficiently use the mechanical properties such as stiffness and strength of the material they have. These materials may be manufactured or natural. Transportation costs influence whether to use local or imported materials. Asphalt-bearing rock was not proving satisfactory. It failed in use and was expensive to import from Europe. De Smedt had the insight to control the stiffness and strength of the asphalt mix by using aggregate with point-to-point contact between the particles. This is a well-graded mix in today's terminology, and de Smedt's patent uses a mix that is not out of range for a mix with a small maximum size of stone. Even in de Smedt's day, it was realized that no natural asphalt was suitable "for all climates and seasons" and that the asphalt must be combined in such a way that it was not brittle in winter or soft in summer (Gillmore 1876). Chemical experimentation was needed. De Smedt trained as a chemist in Europe; European chemistry was more advanced than American (Lucier 2008). De Smedt had the insight to mix the stiff TLA with a flux to reduce the viscosity of the asphalt so it could be mixed with the aggregates. However, he also recognized that this mixture would have performance issues when the pavement was used by the steel rimmed wagons

of the time. His use of grahamite, from West Virginia, to regain the stiffness of the binder was important to the success of his pavements and laid the foundation for modern asphalt pavement mix design and construction.

## REFERENCES

- Anonymous. 1866. *Scientific Intelligence II. Mineralogy and Geology*. American Journal of Science and Arts, November 1866: **42**, (126): 420 and 421.
- Anonymous. 1869. *Asphalt Pavements*. New York Times October 9, 1869.
- Anonymous. 1873. *Asphalt Pavements*. Popular Science Monthly **2** March 1873: 609-613.
- Anonymous. 1875. The Asphalt Pavement on Fifth Avenue Opposite Worth Monument New York. Francis & Loutrel, New York. 71p.
- Anonymous. 1885. *Asphalt Pavements*. Engineering News and American Contract Journal, **XIII** February 28, 1885: 129.
- Anonymous. 1906. District of Columbia Appropriation Bill. Statements before the Subcommittee of the Committee on Appropriation, United States Senate, on The Bill (H.R. 18198) Making Appropriation to Provide for the Expenses of the Government of the District of Columbia for the Fiscal Year Ending June 30, 1907, and for Other Purposes. Government Printing Office, Washington, D.C.
- Bartlett, Larry. 2010. *Ritchie Mines*, eWV: The West Virginia Encyclopedia, [www.wvencyclopedia.org/articles/86](http://www.wvencyclopedia.org/articles/86), accessed 3/27/2013.
- Baskerville, Charles. 1909. *Economic Possibilities of American Oil Shales-I*. The Engineering and Mining Journal N, **LXXXVIII** No. 4, July 24, 1909: 149-154.

- Byrne, Austin T. 1908. *A Treatise on Highway Construction, 5<sup>th</sup> edition Revised and Enlarged*. John Wiley & Sons, New York; Chapman & Hall, London. 1040p.
- De Smedt, Edward Joseph. 1870. *Improvement in Asphalt Roads and Pavements*, United States Patent Office No. 103,582, dated May 31, 1870.
- De Smedt, Edward. 1893. *Asphalt and Asphalt Paving*, Paving and Municipal Engineering **5**:(5)(November): 205-208.
- Dyni, John R. 2005. *Geology and Resources of Some World Oil-Shale Deposits*, United States Geological Survey Scientific Investigations Report 2005-5294. 42p.
- Fontaine, William M. 1873. *Art. XLV-Notes on the West Virginia Asphaltum Deposit*, American Journal of Science and Arts, 3<sup>rd</sup> Series **VI**, (Whole Number **CVI**): 409-416.
- Gibson, Nelson, Xicheng Qi, Aroon Shenoy, Ghazi Al-Khateeb, M. Emin Kutay, Adrian Andriescu, Kevin Stuart, Jack Youtcheff and Thomas Harman. 2012. *Performance Testing For Superpave and Structural Validation*, FHWA-HRT-11-045. 253p.
- Gillespie, William Mitchell. 1849. *Manual of the Principals and Practice of Road-making: Comprising the Location, Construction and Improvement of Roads (Common, Macadam, Paved, Plank, etc.) and Rail-roads*, A.S.Barnes, New York. 372p.
- Gillmore, Q.A. 1876. *A Practical Treatise on Roads, Streets, and Pavements*, D. Van Nostrand, New York. 258p.
- Glenn, William. 1895. *Mine-Explosions Generated by Grahamite Dust*, Transactions of the American Institute of Mining Engineers **XXIV**: 195-207.
- Gray, J.E. and J.E. Bell. 1964. *Stone Sand*, National Crushed Sand Association Engineering. Bulletin 13. 70p.
- Green, Anna Katherine. 1881. *The Sword of Damocles*, New York, G.P. Putnam's Sons.
- Grimsley, G.P. 1910. *Pleasants, Wood, and Ritchie Counties*. West Virginia Geological and Economic Survey (county report series), Morgantown, West Virginia. 352p.
- Hodge, Janet. 1992. *Ritchie Mines; A Beginning in History*. <http://files.usgwarchives.net/wv/Ritchie/history/ritchmin.txt>, accessed 3/27/2013 (apparently taken from an article in the Ritchie Gazette, Harrisville, West Virginia in 1992)
- Holley, I.B. 2003. *Blacktop: How Asphalt Paving Came to the United States*. Technology and Culture, The Johns Hopkins University Press, **44**(4): 703-733.
- Lake Asphalt of Trinidad and Tobago. 2013. *History of the Company*, [http://www.trinidadlakeasphalt.com/pdf/history\\_page.pdf](http://www.trinidadlakeasphalt.com/pdf/history_page.pdf), accessed 2/7/2014.
- Law, Henry and D. Kinnear Clark. 1877. *The Construction of Roads and Streets*. Crosby Lockwood & Co., London. 345p.
- Lay, M.G. 1992. *Ways of the World: A History of the World's Roads and of the Vehicles That Used Them*. Rutgers University Press, New Brunswick, New Jersey. 401p.
- Lesley, J.P. 1863. Professor Lesley communicated a notice of a remarkable coal mine or Asphalt vein, cutting the horizontal Coal-measures of Wood County, Western Virginia. Proceedings of the American Philosophical Society, **9**:(69) (January 1863): 184-204.

- Lowther, Minnie Kendall. 1911. History of Ritchie County, Wheeling News Litho Co., Wheeling, West Virginia. 681p.
- Lowther, Minnie Kendall, *History of Ritchie County*. 1916. E-book: Lucier, Paul. 2008. Scientists and Swindlers: Consulting on Coal and Oil in America 1820-1890. Johns Hopkins Press, Baltimore. 426p.
- Macfarlane, James. 1875. *The Coal-Regions of America: Their Topography, Geology, and Development* 3<sup>rd</sup> Ed. D. Appleton and Company, New York. 695 p.
- Murphy, Jody. 2008. *Asphalt Mines Largely Unremembered*. [http://www.newsandsentinel.com/page/content\\_detail/id/508162.html](http://www.newsandsentinel.com/page/content_detail/id/508162.html), accessed 3/27/2013, (apparently from the Parkersburg, West Virginia, News and Sentinel August 13, 2008).
- North, Edward P. 1879. *The Construction and Maintenance of Roads*. Transactions of the American Society of Civil Engineers, **VIII**, (whole number *CLXXX*): 95-114.
- Old Newark website. 2013. [www.newarkphotos.com/publicbuildings/thumbnails.php?album=14](http://www.newarkphotos.com/publicbuildings/thumbnails.php?album=14), accessed 9/24/13.
- Pepper, Brooks. 1939. *Cairo and Kanawha Valley Narrow Gauge Marks Interesting Period in State History*. <http://filebox.vt.edu/users/jopjacks2/pubs/RitchieGazetteExtracts1939.htm>, accessed 3/27/2013, (apparently and extract from the Ritchie Gazette, February 17, 1939).
- Poore, Ben Perley, ed. 1872. Message From the President of the United States to the Two Houses of Congress at the Commencement of the Second Session of the Forty-Second Congress. With the Reports of the Heads of Departments and Selections From Accompanying Documents, Government Printing Office, Washington, 785p.
- Renninger, F.A. and F.P. Nichols, Jr. 1968. *Research in the Mineral Aggregate Industry*, Society of Mining Engineers of AIME, Preprint Number 68-H-331. 11p.
- Richardson, Clifford. 1905. *The Modern Asphalt Pavement* 1<sup>st</sup> ed. John Wiley & Sons, New York; Chapman & Hall, London. 580p.
- Richardson, Clifford. 1912. *The Modern Asphalt Pavement* 2<sup>nd</sup> ed. John Wiley & Sons, New York; Chapman & Hall, London. 629p.
- Ripley, George and Charles A. Dana. 1868. *The New American Cyclopaedia*. D. Appleton and Company, New York, **13**: 55-57.
- Ripley, George and Charles A. Dana. 1875. *The American Cyclopaedia*. D. Appleton and Company, New York, **XIII**: 191-194.
- Ritchie County Tourism and Visitor's Bureau, Inc. 2009. *Wildlife Management Areas*. [www.visitritchiecounty.com/page.php?id=82](http://www.visitritchiecounty.com/page.php?id=82), accessed 3/27/2013.
- Wood, David Muir. 2012. *Civil Engineering: A Very Short Introduction*. Oxford University Press. 143p.
- WV Geologic Survey, County Surveys & Maps for Pleasants, Wood and Richie Counties. 1910. available as a free e-book: <https://archive.org/details/peasantswoodritc00westuoft>

## Microbial isolates in diseased fishes, primarily smallmouth bass (*Micropterus dolomieu*), within the Chesapeake Bay drainage in 2009-2011.

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### ABSTRACT

During the spring and summers of 2009–2011, our laboratory examined diseased fishes, primarily smallmouth bass *Micropterus dolomieu*, from a number of sites from the South Branch Potomac River, WV, Susquehanna River, PA, and the Monocacy River, MD. Fish were grossly examined and necropsied, with lesions and tissues assayed for bacterial agents and viruses. Fish were also examined histopathologically. Although impossible to assess in the open waters, mortalities were apparently ongoing and chronic, as indicated by high prevalences and varying severity of external body surface lesions. Sixty percent (39/65) of the fish examined in 2010 and 2011 had external lesions. Of the 57 young-of-year (y-o-y) smallmouth bass examined from the Susquehanna River, 32 (56.1%) had lesions. *Flavobacterium columnare* and *Aeromonas veronii* bv. *sobria* were the most common bacterial pathogens recovered. *Plesiomonas shigelloides* was also recovered from 11 (19.3%) y-o-y smallmouth bass internal tissue homogenates. *Aeromonas salmonicida* subsp. *salmonicida*, a significant primary pathogen to salmonid fish species, was recovered from the lesions of 2/6 (33%) adult smallmouth bass from the Monocacy River, MD in May 2009, while *F. columnare* was recovered from the kidney of one of these two fish. Largemouth bass virus DNA was detected by qPCR in 39/57 (68.4%) fish from the Susquehanna River, PA in 2010, with a mean of  $6.11 \times 10^6$  VGE/g. Histopathology showed a diffuse granulomatous response throughout the spleens and in the gut submucosa and/or the surrounding connective tissue with eosinophilic granular cells and fibrosis adjacent to the granulomas. In some cases, the inflammation infiltrated the muscularis.

### INTRODUCTION

Fish die-offs within the Chesapeake Bay drainage involving infectious agents and various host species have been previously documented (Snieszko *et al.* 1964; Bullock 1972; Colwell *et al.* 1977; Okpokwasili 1991; Kane *et al.* 1998; Blazer *et al.* 2010). Although well-recognized etiological agents have been recovered, these events have involved a suite of environmental parameters to precipitate disease expression sometimes from otherwise opportunistic pathogens (Snieszko 1974; Seliger *et al.* 1985; Starliper *et al.* 1992; Wedemeyer 2001). Compromising environmental perturbations include atypical water temperatures, low dissolved oxygen, reduced or increased water flows, and pollutants that cause immunosuppression.

During the spring and summers of 2009–2011, our laboratory provided assistance to federal and state agencies from West Virginia, Maryland and Pennsylvania with fish disease diagnostic cases and scheduled surveys of fishes from sites with recent disease histories. We received or collected fish for examinations from sites in the South Branch Potomac River, WV, Susquehanna River, PA, and Monocacy River, MD. Fish from the South Branch were randomly collected for fish health assessments as major mortalities occurred in this area during previous years (Blazer *et al.* 2010). Fish from the Monocacy River were collected during a single die-off event involving adult bass and other species. In the Susquehanna River, various species of fish, predominately young-of-year (y-o-y) smallmouth bass *Micropterus*



*dolomieu*, were noted with skin lesions and mortality; therefore, fish from several collection sites on the river were examined. Based on observations and suspected host specificity, our efforts were primarily towards smallmouth bass. The fish were grossly examined and necropsied, and tissues were assayed for bacterial agents and viruses.

## MATERIALS AND METHODS

Fish were collected from ten different sites from rivers in West Virginia, Maryland and Pennsylvania (Tables 1 and 2; Figure 1). Live fish were collected using a boat and either hand-netting moribund individuals or standard electrofishing methods. Although smallmouth bass was the targeted species, additional affected species, namely redhorse suckers *Moxostoma carinatum*, largemouth bass *M. salmoides*, and green sunfish *Lepomis cyanellus* were also examined. Fish were transferred to live-well holding tanks with source-river water and transported to the National Fish Health Research Laboratory in Leetown, WV. The time between collection and laboratory examinations did not exceed 5 h. The tanks were insulated to maintain temperature and the fish were supplied with oxygen using an air stone and aquarium pump. Upon receipt at the laboratory, the fish were euthanized with a lethal dose of tricaine methanesulfonate (250 mg/L; Argent Chemical Laboratories, Redmond, WA).

Fish were examined grossly for lesions. Bacteria were cultured from the leading margins of the lesions when present on the fish and/or the skin/mucus using a sterile inoculating loop. For the diagnostic sampling in 2009, this material was used to streak-plate inoculate the following bacteriological media: tryptic soy agar (TSA; Becton, Dickinson and Company, Sparks, MD), R2A (Becton, Dickinson and Company, Sparks, MD) and cytophaga agar (Anacker and Ordal 1959). The body cavity of each fish was aseptically opened and kidney tissue was used to streak-plate inoculate the same media (TSA, R2A, cytophaga agar). Paired sets of plates were incubated at 16°C and 23°C.

In 2010 and 2011, the procedures for gross examination and streak-plate inoculations of lesions were the same as previously described. The procedure to recover bacteria from internal tissues was different and a more extensive battery of bacteriological media was employed for both the external lesions and internal tissues. Once the body cavity was aseptically opened, kidney, spleen and swim bladder tissues were aseptically removed and placed as single fish pools into Hanks balanced salt solution (Sigma-Aldrich, Co., St. Louis, MO) to achieve a 1:10 dilution (w/v). Samples were homogenized using a Ten Broek homogenizer and the particulate material was pelleted by centrifugation at 500 x g for 10 min at 4 °C (Beckman-Coulter Allegra 6R, Brea, CA). Ten microliters of supernatant from each homogenate was applied to each plate of the suite of media, and then plates were streak-inoculated using a sterile loop. Plates were incubated at 23 °C.

Both non-selective and selective primary isolation media were employed in 2010-2011 to recover many possible bacterial pathogens. Based on the histopathology results from the diagnostics done in 2009, we suspected a significant involvement by the common fish pathogen *Flavobacterium columnare* as well as the possible involvement of *Francisella* sp.; therefore, the following media were used in addition to TSA, R2A and cytophaga agar: selective columnaris agar (Hawke and Thune 1992), S#2 developmental medium (C. E. Starliper, unpublished data), blood agar base (Becton, Dickinson and Company, Sparks, MD) supplemented with 0.1% l-cysteine (Sigma-Aldrich, Co., St. Louis, MO) and 1% glucose (Sigma-Aldrich, Co., St. Louis, MO; Mauel 2010), Thayer-Martin (Becton, Dickinson and Company, Sparks, MD), cystine-heart agar (CHA; Becton, Dickinson and Company, Sparks, MD) supplemented with 1% bovine hemoglobin (Becton, Dickinson and Company, Sparks, MD), with and without 100 u polymyxin B (Sigma-Aldrich, Co., St. Louis, MO) and 50 ug/mL ampicillin (Sigma-Aldrich, Co., St.

Louis, MO; Soto *et al.* 2009), CHA with 5% rabbit blood (Hemostat Laboratories, Dixon, CA) with and without the same concentrations of polymyxin B and ampicillin as previously described, and CHA with 5% sheep blood (Hemostat Laboratories, Dixon, CA) with and without the same concentrations of polymyxin B and ampicillin as before (Ottem *et al.* 2009). Following incubation, the plates were inspected daily for bacterial growth for up to three weeks. Single bacterial colonies representing the colony morphologies recovered were transferred to fresh homologous media to develop pure cultures for identifications.

The bacterial cultures were characterized using standard bacterial classification procedures and previously published line data for comparisons (Griffin 1992; Koneman *et al.* 1992; Holt *et al.* 1994; Janda and Abbott 1998; MacFaddin 2000; Miñana-Galbis *et al.* 2002; 2004; 2007; 2010; Abbott *et al.* 2003; Murray *et al.* 2003; Salerno *et al.* 2007; Demarta *et al.* 2008; Martínez-Murcia *et al.* 2008; Beaz-Hidalgo *et al.* 2009; 2010).

The kidney-spleen-swim bladder homogenates were used to inoculate FHM or BF-2 cell monolayers. The SP1 cell line was inoculated for the culture of putative chlamydial/rickettsial agents. Cells were grown to confluence in 24-well plates and incubated at 20 or 25 °C. Cells were examined daily for 10 days using an inverted microscope (Axiovert 25, Zeiss). A blind passage (1:10 dilution) was performed for all cultures not exhibiting a cytopathic effect after the primary incubation and observed for another 10 days.

Quantitative PCR was used to determine LMBV titers in pooled tissue samples (kidney, spleen and swim bladder) using the method described by Getchell *et al.* (2007); however, it was conducted as a SYBR green assay. In short, total DNA was extracted from pooled tissue samples using a DNeasy Blood and tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's protocols. The qPCR assay was run on a Qiagen Rotor-Gene 6000 (Qiagen).

Seven serial dilutions of LMBV clone (kindly supplied by Dr. R. Getchell, Cornell University) were used to generate a standard curve for each qPCR run. The modified qPCR protocol was as follows. A Promega GoTaq qPCR kit was used for all reactions. Each reaction contained 2 ul of template DNA, 0.3 µM of the forward primer 398F (5'-TGATTGGCAACACTAGCGATCT-3'), and 0.3 µM of the reverse primer 459R (5'-CCTAGCTCCTGCTTGATCGG-3'). All reactions were run in duplicate as 20-µl volumes. The PCR conditions were as follows: 95 °C for 10 min, then 50 cycles of 95 °C for 15 s and 60 °C for 30 s. Data were analyzed using Rotor-Gene Q - Pure Detection; Version 1.7 (Build 94) software.

## RESULTS

The diagnostic cases completed in 2009 showed that the primary fish species affected by the diseases and die-offs was smallmouth bass (Table 1). Other species affected by the disease apparently to lesser degrees were largemouth bass, redhorse suckers and green sunfish. The fish presented with a variety in external pathology ranging from small foci of necrosis, which we speculate were early in the progression of lesion development, to discoloration with significant loss of scales to extensive necrosis covering large portions of the body (Figures 2-5). Some of the fish had focal sites of necrosis surrounded by unique, wide margins of discoloration which were presumably the leading edges of the lesions (Figure 4). Other fish were affected with significant caudle peduncle involvement including complete erosion of the caudle fin (Figures 5a and 5b). There was no apparent consistency in morphology of the lesions or predilection for a particular location on the fish. Significant pathology was observed of fish from all collection sites.

*Flavobacterium columnare* was recovered from lesions of y-o-y smallmouth bass and golden shiners from the Harrisburg, PA site and from golden shiners from the

Williamsport, PA site (Table 1). This pathogen was also recovered from the kidney of one adult smallmouth bass from the Monocacy River, MD and from kidneys of 6/7 (85.71%) golden shiners from the two Susquehanna River sites. *Aeromonas salmonicida* subsp. *salmonicida* was recovered from the lesions of 2/6 smallmouth bass from the Monocacy River in May 2009; a co-infection with *F. columnare* was also diagnosed from the kidney of one of these two fish. *Aeromonas veronii* bv. *sobria* was recovered from lesions from all of the diagnostic cases (Table 1). Additional bacteria recovered from lesions and kidneys from fish examined during the diagnostic cases included other *Aeromonas* spp., *Staphylococcus* sp., *Shewanella putrefaciens*, *Plesiomonas shigelloides* and coliform bacteria.

Sixty-five fish were examined from the surveys conducted during the summers of 2010 and 2011; 62 were smallmouth bass, all but five being y-o-y smallmouth bass (Table 2). The collection sites were identified based on results from the diagnostic cases in 2009, known spawning areas for smallmouth bass and recent accounts in which diseased and/or dying smallmouth bass were observed (Figure 1). Of the 57 y-o-y smallmouth bass examined, 32 (56.1%) had lesions, and *F. columnare* was recovered from 24 of these (75.0%). *Flavobacterium columnare* was also recovered from two internal tissue homogenates from y-o-y smallmouth bass collected from the Danville site. In addition to *F. columnare*, *A. veronii* bv. *sobria* was frequently recovered from y-o-y smallmouth bass, from 20 (62.5%) of the lesions and from 16 of 57 (28.1%) internal tissue homogenates. *Plesiomonas shigelloides* was recovered from 11 (19.3%) y-o-y smallmouth bass internal tissue homogenates and from the lesion of one of these fish, from Clemson Island in 2010. Other bacteria recovered from lesions and internal tissue homogenates included *A. hydrophila*, *A. popoffii* and *S. putrefaciens*. *Francisella* sp. was not recovered from any of the fish.

Eight fish other than y-o-y smallmouth bass were collected during the surveys, five year-class-one smallmouth bass and one each of y-o-y largemouth bass, redhorse sucker and green sunfish (Table 2). Seven of these fish had lesions and *F. columnare* was recovered from six (85.7%), including the four year-class-one smallmouth bass from the Juniata River site. *Aeromonas veronii* bv. *sobria* was also recovered from all of the lesions (100%). However, *F. columnare* was not recovered from any internal tissue homogenates and *A. veronii* bv. *sobria* was recovered from only one, which was the y-o-y largemouth bass from the Clemson Island site in June 2010. *Plesiomonas shigelloides* was recovered from one internal tissue homogenate, also the y-o-y largemouth bass, but not from any lesions.

Confirmation of the *F. columnare* isolates was accomplished with the characteristic rhizoid colony morphology, chondroitinase-positive and other specific test results (Griffin 1992). Genus level identifications of *Aeromonas* spp. were accomplished with the following criteria: Gram-negative rods, fermentation of glucose, oxidase positive, reduction of nitrates, and resistance to 100 µg 2,4-diamino-6,7-diisopropyl-pteridine (Vibriostat; Sigma-Aldrich, Co., St. Louis, MO) per mL of Mueller Hinton medium (Becton, Dickinson and Company, Sparks, MD). Motility was determined using the hanging-drop procedure, which distinguished the fish pathogen *A. salmonicida* subsp. *salmonicida*, which is non-motile, from other *Aeromonas* species. Additional testing characterized the isolates to species. For example, *A. veronii* bv. *sobria* was differentiated from other *Aeromonas* spp. by gas produced from glucose, positive reactions from Voges-Proskauer, arginine decarboxylase/dehydrolase, sucrose and d-mannitol, and negatives from ornithine decarboxylase, L-arabinose, esculin hydrolysis and inositol. Diagnostic test results used for *P. shigelloides* included alkaline/acid reaction with no gas production on triple sugar iron medium (TSI), resistance to Vibriostat, oxidase-positive,

and positives for lysine decarboxylase, ornithine decarboxylase and arginine decarboxylase/dehydrogenase. Identification of *S. putrefaciens* was essentially diagnostic with a non-fermentative TSI reaction, but with vigorous production of H<sub>2</sub>S gas within 8-12 h.

The results of LMBV detection and the predominant bacteria recovered from fish in the Susquehanna River, 2010 are summarized in Table 3. Largemouth bass virus was present in fish from all collection sites with 39 of 57 (68.4%) fish positive by qPCR. Cytopathic effect (CPE) was observed in FHM and BF-2 cells at 20 or 25 °C, and the CPE-positives were confirmed as LMBV by endpoint PCR. The mean LMBV detected from the collection sites ranged from  $2.80 \times 10^6$  VGE/g (viral genome equivalents per gram) from the Juniata River to  $1.06 \times 10^7$  VGE/g from Liverpool. The overall mean for all fish sampled in 2010 was  $6.11 \times 10^6$  VGE/g. The qPCR was more sensitive than tissue culture for detecting the presence of the virus. Fish were typically tissue culture negative when estimated genome copies were less than  $5 \times 10^4$  VGE/g. *Aeromonas veronii* bv. *sobria* was recovered from 14 (24.6%) internal tissue homogenates of which 13 (92.9%) were also positive for LMBV. *Plesiomonas shigelloides* was recovered from 11 (19.3%) internal tissue homogenates and 10 (90.9%) of these were positive for LMBV. There was no evidence for growth of chlamydia or rickettsia in inoculated SP1 cells.

Histopathology from 2010 of internal tissues from y-o-y smallmouth bass from the Danville and Clemson Island sites showed that 9/14 and 1/7 fish, respectively, developed a diffuse granulomatous response in the submucosa of their gut and/or the surrounding connective tissue. Eosinophilic granular cells (EGCs) and fibrosis were observed around or near the granulomas. In some cases, the inflammation infiltrated the muscularis. In 2011, 4/9 y-o-y smallmouth bass from the Shady Nook site displayed a diffuse granulomatous response throughout their spleens. The granulomas

consisted of necrotic centers with ceroid macrophages and hemorrhage. Although special stains including Acid Fast (for Mycobacteria), Gram (for bacteria), Periodic Acid Schiff (for fungi) and Giemsa (for parasites) were applied to slides, the cause of the granulomatous inflammation was not apparent.

## DISCUSSION

The high percentages of fish with lesions and varying degrees of lesion severity were suggestive of an active, chronic disease scenario with likely involvement of an infectious agent(s). This scenario contrasts with what would be anticipated from an acute disease expression, such as that caused by a lethal water quality event. An acute exposure, for example, to a chemical is typically characterized with mortality within a relatively short timeframe, minimal to no lesion expression and a wider host range, perhaps not limited to fishes. In the present study, smallmouth bass was the primary affected host, and in the survey done in 2010 and 2011 in the Susquehanna River, y-o-y smallmouth bass were particularly affected. Although host specificity alone is not indicative of a particular disease, it is often associated with infectious causes. Host specificity may be less informative in free-ranging fishes when fish population distribution data at the sampling sites are not known.

It is not uncommon to recover certain *Aeromonas* spp. or *F. columnare* from diseased fishes, including warm- and cool-water species, and wild or hatchery-reared populations (Hawke and Thune 1992; Joseph and Carnahan 1994). Both of these bacteria are generally recognized as ubiquitous inhabitants of freshwater aquatic environments (Hawke and Thune 1992; Holmes *et al.* 1996) and can be recovered from healthy fishes and other aquatic animals including invertebrates (Starliper *et al.* 1998; 2008). Within the Chesapeake drainage, columnaris disease (i.e., *F. columnare*) will typically begin to occur in spring as water temperatures rise, especially 13 °C and above. Similarly, *Aeromonas* spp. are opportunistic and cause

diseases to fishes that might be compromised from host stressors including increased water temperatures, reduced dissolved oxygen levels, or exposure to sublethal concentrations of immunosuppressive chemicals. *Aeromonas veronii* bv. *sobria* accounted for the majority of *Aeromonas* spp. recovered from both the lesions (25/36; 69.4%) and internal tissue homogenates (16/62; 25.8%) from smallmouth bass from the Susquehanna River (Table 2). *Flavobacterium columnare* was recovered from an even greater number of smallmouth bass lesions (28/36; 77.8%). Co-infections of *A. veronii* bv. *sobria* and *F. columnare* were detected from 21/36 (58.3%) of the lesions. There was only one smallmouth bass in which both of these bacteria were recovered from the same internal tissue homogenate from the Danville site in 2010 (Table 2).

There was a high prevalence of *F. columnare* from hosts other than smallmouth bass, including golden shiners (6/7; 85.71%) and green sunfish (1/2; Table 1). Subsequently, *F. columnare* was recovered from lesions of the white sucker and largemouth bass in 2010 (Table 2). *Flavobacterium columnare* is known to have a broad host range and affects all ages from fry to adult, and cultured and feral populations (Bullock *et al.* 1971). Also, this pathogen frequently occurs as a co-infection with other bacterial pathogens, namely *Aeromonas* spp. (Starliper and Schill 2011).

Blazer *et al.* (2010) noted a number of contributing factors to the overall poor health of centrarchid fish populations, particularly smallmouth bass, within the Potomac River drainage. These factors include pathologic changes such as intersex, gill pathology and increased macrophage aggregates, all of which may indicate exposures to environmental contaminants. Opportunistic pathogens such as *F. columnare*, *Aeromonas* spp., parasites and LMBV were frequently recovered or detected from these compromised hosts. High prevalences of these same pathogens in the present study may also be indicative of poor

health possibly related to environmental input. The high recovery of *P. shigelloides* and the high prevalence of LMBV, which are considered opportunistic pathogens, may also indicate poor health in affected populations. Schramm *et al.* (2006) showed the stress to largemouth bass in live-well holding tanks during fishing tournaments was significantly reduced by improving the holding conditions. However, post-release mortality of fish held for five days was not significantly reduced with the improved live-well holding conditions. The authors speculated this was due in part to LMBV through facilitation of viral transmission in addition to immunosuppression from the stresses of capture and handling. The high prevalences of co-infections of LMBV with both *A. veronii* bv. *sobria* and *P. shigelloides* in the present study, were also shown by Schramm *et al.* (2006) with co-infections of LMBV with *F. columnare* and *Aeromonas* spp. in largemouth bass in fishing tournaments in the southern United States.

The relatively high percent recovery of *P. shigelloides* from the internal tissue homogenates from the Susquehanna River in 2010 was noteworthy. This bacterium is distributed in the aquatic environment and has been recovered from fishes, water and invertebrates (Arai *et al.* 1980; Miller *et al.* 2006; Salgado-Miranda *et al.* 2010; Starliper *et al.* 2011); however, it is not recognized as a primary pathogen to free-ranging fish populations. Although uncommon, *P. shigelloides* can cause human diseases primarily as a cause of gastroenteritis or infections to immunocompromised patients (Arai *et al.* 1980; Meng *et al.* 2012; Reinhardt and George 1985). In conclusion, the results of these surveys/ diagnostic cases indicate multiple microbial pathogens are contributing to morbidity and mortality, and that environmental factors may be leading to immunocompromised fishes.

#### **Conflict of interest statement**

*Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.*

## LITERATURE CITED

- Abbott, S.L., W.K.W. Cheung, and J.M. Janda. 2003. *The genus Aeromonas: biochemical characteristics, atypical reactions, and phenotypic identification schemes*. J. Clin. Microbiol. **41**(6): 2348-2357.
- Anacker, R.L. and E.J. Ordal. 1959. *Studies on the Myxobacterium Chondrococcus columnaris. I. Serological typing*. J. Bacteriol. **78**(1): 25-32.
- Arai, T., N. Ikejima, T. Itoh, S. Sakai, T. Shimada, and R. Sakazaki. 1980. *A survey of Plesiomonas shigelloides from aquatic environments, domestic animals, pets and humans*. J. Hyg. **84**(2): 203-211.
- Beaz-Hidalgo, R., A. Alperi, M.J. Figueras, and J.L. Romalde. 2009. *Aeromonas piscicola sp. nov., isolated from diseased fish*. Syst. Appl. Microbiol. **32**(7): 471-479.
- Beaz-Hidalgo, R., A. Alperi, N. Buján, J.L. Romalde, and M.J. Figueras. 2010. *Comparison of phenotypical and genetic identification of Aeromonas strains isolated from diseased fish*. Syst. Appl. Microbiol. **33**(3): 149-153.
- Bullock, G.L. 1972. *Studies on selected myxobacteria pathogenic for fishes and on bacterial gill disease in hatchery-reared salmonids*. Technical Paper No. 60. U.S. Fish and Wildlife Service, Washington, DC. 30 pp.
- Bullock, G.L., D.A. Conroy, and S.F. Snieszko. 1971. *Bacterial diseases of fishes* in S.F. Snieszko and H.R. Axelrod (eds.), Book 2A in Diseases of Fishes, 151 pp. TFH Publications. Neptune City, NJ.
- Blazer, V.S., L.R. Iwanowicz, C.E. Starliper, D.D. Iwanowicz, P. Barbash, J.D. Hedrick, S.J. Reeser, J.E. Mullican, S.D. Zaugg, M.R. Burkhardt, and J. Kelble. 2010. *Mortality of centrarchid fishes in the Potomac drainage: survey results and overview of potential contributing factors*. J. Aquat. Anim. Health. **22**(3): 190-218.
- Colwell, R.R., J. Kaper, and S.W. Joseph. 1977. *Vibrio cholerae, Vibrio parahaemolyticus, and other Vibrios: occurrence and distribution in Chesapeake Bay*. Science. **198**(4315): 394-396.
- Demarta, A., M. Küpfer, P. Riegel, C. Harf-Monteil, M. Tonolla, R. Peduzzi, A. Monera, M.J. Saavedra, and A. Martínez-Murcia. 2008. *Aeromonas tecta sp. nov., isolated from clinical and environmental sources*. Syst. Appl. Microbiol. **31**(4): 278-286.
- Getchell, R.G., G.H. Groocock, V.L. Schumacher, S.G. Grimmett, G.A. Wooster, and P.R. Bowser. 2007. *Quantitative polymerase chain reaction assay for largemouth bass virus*. J. Aquat. Anim. Health. **19**(4): 226-233.
- Griffin, B.R. 1992. *A simple procedure for identification of Cytophaga columnaris*. J. Aquat. Anim. Health. **4**(1): 63-66.
- Hawke, J.P. and R.L. Thune. 1992. *Systemic isolation and antimicrobial susceptibility of Cytophaga columnaris from commercially reared channel catfish*. J. Aquat. Anim. Health. **4**(2): 109-113.
- Holmes, P., L.M. Niccolls, and D.P. Sartory. 1996. *The ecology of mesophilic Aeromonas in the aquatic environment* in B. Austin, M. Altwegg, P.J. Gosling and S.W. Joseph (eds.), The Genus *Aeromonas*, pp. 127-150. John Wiley and Sons Ltd. West Sussex, England.
- Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (eds.). 1994. *Bergey's Manual of Determinative Bacteriology, 9<sup>th</sup> Edition*. Williams and Wilkins. Baltimore, MD. 787 pp.

- Janda, J.M. and S.L. Abbott. 1998. *The Enterobacteria*. Lippincott-Raven Publishers. Philadelphia, PA. 387 pp.
- Joseph, S.W. and A. Carnahan. 1994. *The isolation, identification, and systematics of the motile Aeromonas species*. Annu. Rev. Fish Dis. **4**: 315-343.
- Kane, A.S., D. Oldach, and R. Reimschuessel. 1998. *Fish lesions in the Chesapeake Bay: Pfiesteria-like dinoflagellates and other etiologies*. MD. Med. J. **37**(3): 106-112.
- Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger, and W.C. Winn, Jr. 1992. *Color atlas and textbook of diagnostic microbiology, fourth edition*. J.B. Lippincott Company. Philadelphia, PA. 1154 pp.
- MacFaddin J.F. 2000. *Biochemical tests for identification of medical bacteria, third edition*. Lippincott Williams and Wilkins. Philadelphia, PA. 912 pp.
- Martínez-Murcia, A.J., M.J. Saavedra, V.R. Mota, T. Maier, E. Stackebrandt, and S. Cousin. 2008. *Aeromonas aquariorum sp. nov., isolated from aquaria of ornamental fish*. Int. J. Syst. Evol. Microbiol. **58**(5): 1169-1175.
- Mauel M.J. 2010. *Francisellosis in AFS-FHS (American Fisheries Society-Fish Health Section) Blue Book: Suggested procedures for the detection and identification of certain finfish and shellfish pathogens*. AFS-FHS. Bethesda, MD.
- Meng, S., J. Xu, Y. Xiong, and C. Ye. 2012. *Rapid and sensitive detection of Plesiomonas shigelloides by loop-mediated isothermal amplification of the jugA gene*. PLoS ONE. **7**(10): e41978.
- Miller, W.A., M.A. Miller, and I.A. Gardner. 2006. *Salmonella spp., Vibrio spp., Clostridium perfringens, and Plesiomonas shigelloides in marine and freshwater invertebrates from coastal California ecosystems*. Microb. Ecol. **52**(2): 198-206.
- Miñana-Galbis, D., M. Farfán, J.G. Lorén, and M.C. Fusté. 2002. *Biochemical identification and numerical taxonomy of Aeromonas spp. isolated from environmental and clinical samples in Spain*. J. Appl. Microbiol. **93**(3): 420-430.
- Miñana-Galbis, D., M. Farfán, M.C. Fusté, and J.G. Lorén. 2004. *Aeromonas molluscorum sp. nov., isolated from bivalve molluscs*. Int. J. Syst. Evol. Microbiol. **54**(6): 2073-2078.
- Miñana-Galbis D., M. Farfán, M.C. Fusté, and J.G. Lorén. 2007. *Aeromonas bivalvium sp. nov., isolated from bivalve molluscs*. Int. J. Syst. Evol. Microbiol. **57**(3): 582-587.
- Miñana-Galbis D., M. Farfán, J.G. Lorén, and M.C. Fusté. 2010. *Proposal to assign Aeromonas diversa sp. nov. as a novel species designation for Aeromonas group 501*. Syst. Appl. Microbiol. **33**(1): 15-19.
- Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Tenover, and R.H. Tenover (eds.). 2003. *Manual of Clinical Microbiology, Volume 1, Eighth Edition*. American Society for Microbiology Press. Washington, D.C. 1212 pp.
- Okpokwasili G.C. 1991. *Aeromonas hydrophila: Variability in biochemical characteristics of environmental isolates*. J. Basic Microbiol. **31**(3): 169-176.
- Ottem, K.F., A. Nylund, E. Karlsbakk, A. Friis-Møller, and T. Kamaishi. 2009. *Elevation of Francisella philomiragia subsp. noatunensis Mikalsen et al. (2007) to Francisella noatunensis comb. nov. [syn. Francisella piscicida*

- Ottem et al. (2008) syn. nov.] and characterization of Francisella noatunensis subsp. orientalis subsp. nov., two important fish pathogens. J. Appl. Microbiol. 106(4): 1231-1243.*
- Reinhardt, J.F. and W.L. George. 1985. *Plesiomonas shigelloides*-associated diarrhea. J. Am. Med. Assoc. **253**(22): 3294-3295.
- Salerno, A., A. Delétoile, M. Lefevre, I. Ciznar, K. Krovacek, P. Grimont, and S. Brisse. 2007. *Recombining population structure of Plesiomonas shigelloides (Enterobacteriaceae) revealed by multilocus sequence typing. J. Bacteriol. 189(21): 7808-7818.*
- Salgado-Miranda, C., E. Palomares, M. Jurado, A. Marin, F. Vega, and E. Soriano-Vargas. 2010. *Isolation and distribution of bacterial flora in farmed rainbow trout from Mexico. J. Aquat. Anim. Health. 22(4): 244-247.*
- Schramm Jr., H.L., A.R. Walters, J.M. Grizzle, B.H. Beck, L.A. Hanson, and S.B. Rees. 2006. *Effects of live-well conditions on mortality and largemouth bass virus prevalence in largemouth bass caught during summer tournaments. N. Am. J. Fish. Manag. 26(4): 812-825.*
- Seliger, H.H., J.A. Boggs, and W.H. Biggley. 1985. *Catastrophic anoxia in the Chesapeake Bay in 1984. Science. 228(4): 70-73.*
- Snieszko, S.F. 1974. *The effects of environmental stress on outbreaks of infectious diseases of fishes. J. Fish Biol. 6(2): 197-208.*
- Snieszko, S.F., G.L. Bullock, E. Hollis, and J.G. Boone. 1964. *Pasteurella sp. from an epizootic of white perch (Roccus americanus) in Chesapeake Bay tidewater areas. J. Bacteriol. 88(6): 1814-1815.*
- Soto, E., J.P. Hawke, D. Fernandez, and J.A. Morales. 2009. *Francisella sp., an emerging pathogen of tilapia, Oreochromis niloticus (L.), in Costa Rica. J. Fish Dis. 32(8): 713-722.*
- Starliper, C.E., E.B. Shotts, and J. Brown. 1992. *Isolation of Carnobacterium piscicola and an unidentified Gram-positive bacillus from sexually mature and post-spawning rainbow trout Oncorhynchus mykiss. Dis. Aquat. Org. 13(3): 181-187.*
- Starliper, C.E., R. Villella, P. Morrison, and J. Mathias. 1998. *Studies on the bacterial flora of native freshwater mussels from the Ohio River. Biomed. Lett. 58(229): 85-95.*
- Starliper, C.E., R.J. Neves, S. Hanlon, and P. Whittington. 2008. *A survey of the indigenous microbiota (Bacteria) in three species of mussels from the Clinch and Holston Rivers, Virginia. J. Shellfish Res. 27(5): 1311-1317.*
- Starliper, C.E., J. Powell, J.T. Garner, and W.B. Schill. 2011. *Predominant bacteria isolated from moribund Fusconaia ebena ebonyshells experiencing die-offs in Pickwick Reservoir, Tennessee River, Alabama. J. Shellfish. Res. 30(2): 359-366.*
- Starliper, C.E. and W.B. Schill. 2011. *Flavobacterial diseases: columnaris disease, coldwater disease and bacterial gill disease in P.T.K. Woo and D.W. Bruno (eds.), Fish Diseases and Disorders, Volume 3: Viral, Bacterial and Fungal Infections, 2<sup>nd</sup> Edition, pp. 606-631. CAB International, Oxfordshire, United Kingdom.*
- Wedemeyer, G.A. (ed.). 2001. *Fish Hatchery Management, Second Edition. American Fisheries Society. Bethesda, MD. 733 pp.*



**Table 1.** Summaries of fish disease diagnostics, primarily smallmouth bass (*Micropterus dolomieu*), in spring/summer 2009 from sites within the Chesapeake Bay drainage. Predominant bacteria recovered and identified from skin-mucus-lesion (S-L-M) and kidney tissue streak-plate inoculations.

Collection date and site	Fishes (no. cultured for bacteria) <sup>1</sup>	Predominant bacteria from S-M-L	Predominant bacteria from kidneys <sup>1</sup>
March 2009 South Branch Potomac River, WV	16 adult SMB	<i>Aeromonas veronii</i> bv. <i>sobria</i> , <i>A. hydrophila</i>	NG
	(16) sucker	<i>Serratia odorifera</i> biotype 2, <i>Staphylococcus</i> sp.	NG
May 2009 Monocacy River, MD	(6) adult SMB	<i>A. salmonicida</i> (2/6 +), <i>A. veronii</i> bv. <i>sobria</i> , <i>Shewanella putrefaciens</i>	<i>Flavobacterium columnare</i> (1/6 +), NG: 5/6
	(1) LMB	<i>A. veronii</i> bv. <i>sobria</i>	NG
July 2009 Susquehanna River, Harrisburg, PA	(18) y-o-y SMB	<i>F. columnare</i> (1/18+), <i>A. schubertii</i> , <i>A. veronii</i> bv. <i>sobria</i> , <i>A. caviae</i> , <i>A. hydrophila</i> , <i>Citrobacter</i> sp., <i>Plesiomonas shigelloides</i>	<i>A. veronii</i> bv. <i>sobria</i> , <i>A. hydrophila</i> , NG: 11/18
	(3) shiners	<i>F. columnare</i> (1/3 +), <i>A. veronii</i> bv. <i>sobria</i> , unidentified Gram-positive	<i>F. columnare</i> (3/3 +), <i>A. veronii</i> bv. <i>sobria</i> , <i>A. schubertii</i>
	(2) sunfish	<i>A. hydrophila</i> , <i>P. shigelloides</i>	<i>F. columnare</i> (1/2 +), <i>P. shigelloides</i> , NG: 1/2
July 2009 Susquehanna River, Williamsport, PA	(24) y-o-y SMB	<i>A. veronii</i> bv. <i>sobria</i> , <i>A. caviae</i> , <i>A. schubertii</i> , <i>Pseudomonas</i> sp.	<i>A. veronii</i> bv. <i>sobria</i> , <i>A. caviae</i> , <i>Serratia</i> sp., <i>Enterobacter</i> sp., NG: 13/24
	(4) shiners	<i>F. columnare</i> (3/4 +)	<i>F. columnare</i> (3/4 +), NG: 1/2

<sup>1</sup>Abbreviations: y-o-y, young of the year; SMB, smallmouth bass (*Micropterus dolomieu*); LMB, largemouth bass (*Micropterus salmoides*); redhorse sucker (*Moxostoma carinatum*); golden shiner (*Notemigonus crysoleucas*); green sunfish (*Lepomis cyanellus*); NG, no bacterial growth.

**Table 2.** Bacteria recovered from Susquehanna River, PA sites within the Chesapeake Bay drainage; fish sampled during the summers of 2010–2011. A battery of bacteriological media was inoculated: lesions were streak-plate inoculated and homogenates of kidney, spleen and swim bladder tissues were drop inoculated.

Collection Date and Site	(Number) and hosts sampled <sup>1</sup>	No. of fish with lesions	Lesions: bacteria recovered and the no. of lesions positive <sup>1</sup>	Internal tissues: bacteria recovered and the no. of homogenates positive <sup>1</sup>
July 13, 2010	(5) 1-yr-old SMB	4/5	<i>Flavobacterium columnare</i> 4/4, <i>Aeromonas veronii</i> bv. <i>sobria</i> 4/4	NG
Juniata River	(2) y-o-y SMB	0/2	N/A	<i>A. veronii</i> bv. <i>sobria</i> 1/1, <i>Plesiomonas shigelloides</i> 1/1, NG 1/2
	(1) sucker	1/1	<i>F. columnare</i> 1/1, <i>A. veronii</i> bv. <i>sobria</i> 1/1	NG
July 19, 2010	(7) y-o-y SMB	4/7	<i>F. columnare</i> 4/4, <i>A. veronii</i> bv. <i>sobria</i> 3/4	<i>A. veronii</i> bv. <i>sobria</i> 1/7, <i>P. shigelloides</i> 2/7
Liverpool	(7) y-o-y SMB	5/7	<i>F. columnare</i> 3/5, <i>A. veronii</i> bv. <i>sobria</i> 2/5, <i>P. shigelloides</i> 1/5, <i>A. hydrophila</i> 2/5, <i>A. popoffii</i> 1/5	<i>P. shigelloides</i> 4/7, <i>A. hydrophila</i> 1/7
Clemson Island	(1) y-o-y LMB	1/1	<i>F. columnare</i> 1/1, <i>A. veronii</i> bv. <i>sobria</i> 1/1	<i>A. veronii</i> bv. <i>sobria</i> 1/1, <i>P. shigelloides</i> 1/1
July 20, 2010	(17) y-o-y SMB	14/17	<i>F. columnare</i> 13/14, <i>A. veronii</i> bv. <i>sobria</i> 11/14	<i>F. columnare</i> 2/17, <i>A. veronii</i> bv. <i>sobria</i> 7/17, <i>P. shigelloides</i> 1/17, <i>A. veronii</i> bv. <i>veronii</i> 1/17
Danville	(1) sunfish	1/1	<i>A. veronii</i> bv. <i>sobria</i> 1/1	NG
August 4, 2010	(16) y-o-y SMB	1/16	<i>A. allosaccharophila</i> 1/1	<i>A. veronii</i> bv. <i>sobria</i> 4/16, <i>P. shigelloides</i> 2/16, <i>Shewanella putrefaciens</i> 1/16
Laceyville	(4) y-o-y SMB	4/4	<i>F. columnare</i> 3/4, <i>A. veronii</i> bv. <i>sobria</i> 2/4	<i>A. veronii</i> bv. <i>sobria</i> 1/4
Shady Nook	(4) y-o-y SMB	4/4	<i>F. columnare</i> 1/4, <i>A. veronii</i> bv. <i>sobria</i> 3/4	<i>A. veronii</i> bv. <i>sobria</i> 1/4
July 21, 2011	(4) y-o-y SMB	4/4	<i>F. columnare</i> 1/4, <i>A. veronii</i> bv. <i>sobria</i> 3/4	<i>A. veronii</i> bv. <i>sobria</i> 1/4
Clemson Island				

<sup>1</sup>Abbreviations: y-o-y, young of the year; SMB, smallmouth bass (*Micropterus dolomieu*); LMB, largemouth bass (*Micropterus salmoides*); redhorse sucker, (*Moxostoma carinatum*); green sunfish (*Lepomis cyanellus*); N/A, not applicable; NG, no bacterial growth.

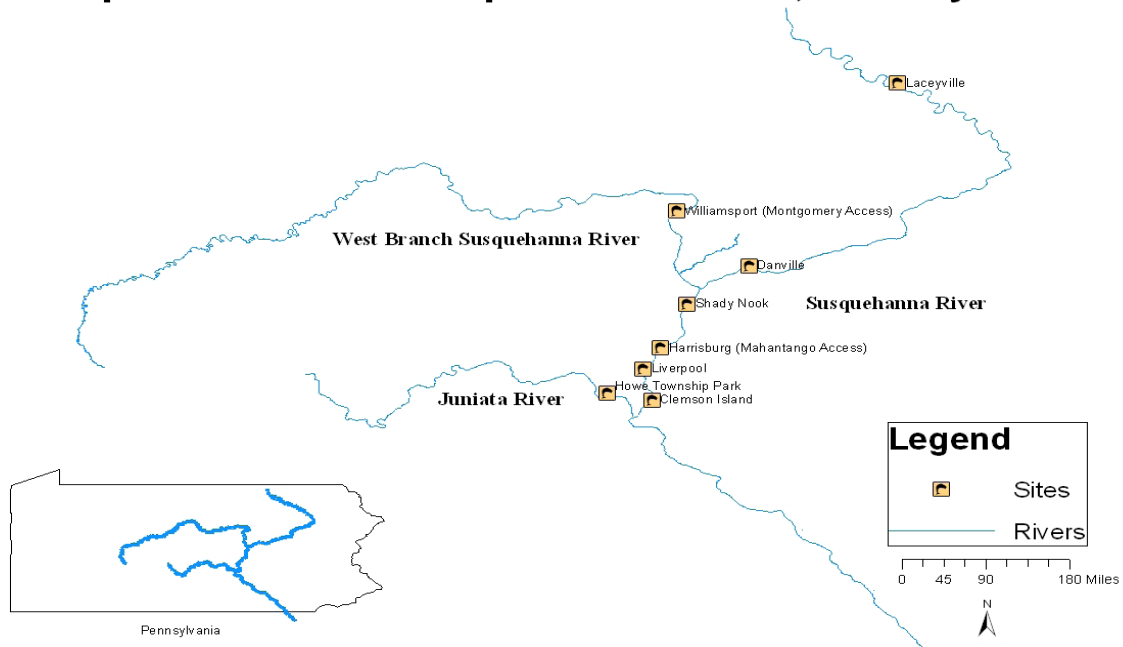
**Table 3.** Predominate fish pathogens recovered and identified from fishes collected from the Susquehanna River, PA, 2010.

Collection	Number of fish with lesions	Lesions positive for A/F/P <sup>1</sup>	Internal tissues positive for A/F/P	Internal tissues positive for LMBV <sup>2</sup>	Mean virus genome equivalents per gram
Juniata River	5/8	5/5/0 of 5	1/0/1 of 8	37.5%; 14.3%	$2.80 \times 10^6$
Liverpool	4/7	3/4/0 of 4	1/0/2 of 7	57.1%; 28.6%	$1.06 \times 10^7$
Clemson Island	6/8	3/4/1 of 6	1/0/5 of 8	75.0%; 71.4%	$5.16 \times 10^6$
Danville	15/18	12/14/0 of 15	7/2/1 of 18	94.4%; 88.2%	$7.58 \times 10^6$
Laceyville	1/16	0/0/0 of 1	4/0/2 of 16	56.3%; 18.8%	$2.83 \times 10^6$
Total	31/57	23/27/1 of 31	14/2/11 of 57	68.4%; 48.1%	$5.61 \times 10^6$

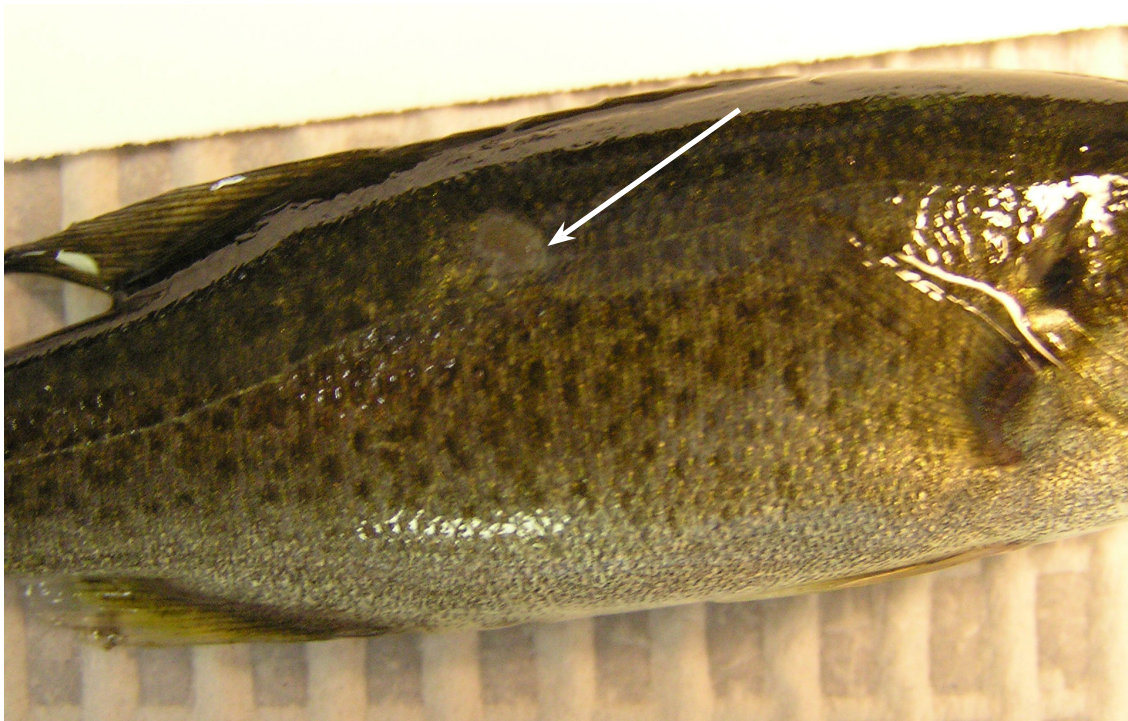
<sup>1</sup>A/F/P = *Aeromonas veronii* bv. *sobria* / *Flavobacterium columnare* / *Plesiomonas shigelloides*.

<sup>2</sup>qPCR positive; tissue culture positive

## Site Locations of Young-of-the-Year Smallmouth Bass Sampled from the Susquehanna River, Pennsylvania



**Figure 1.** Site locations of young-of-the-year smallmouth bass sampled from the Susquehanna River, Pennsylvania, 2009-2011.



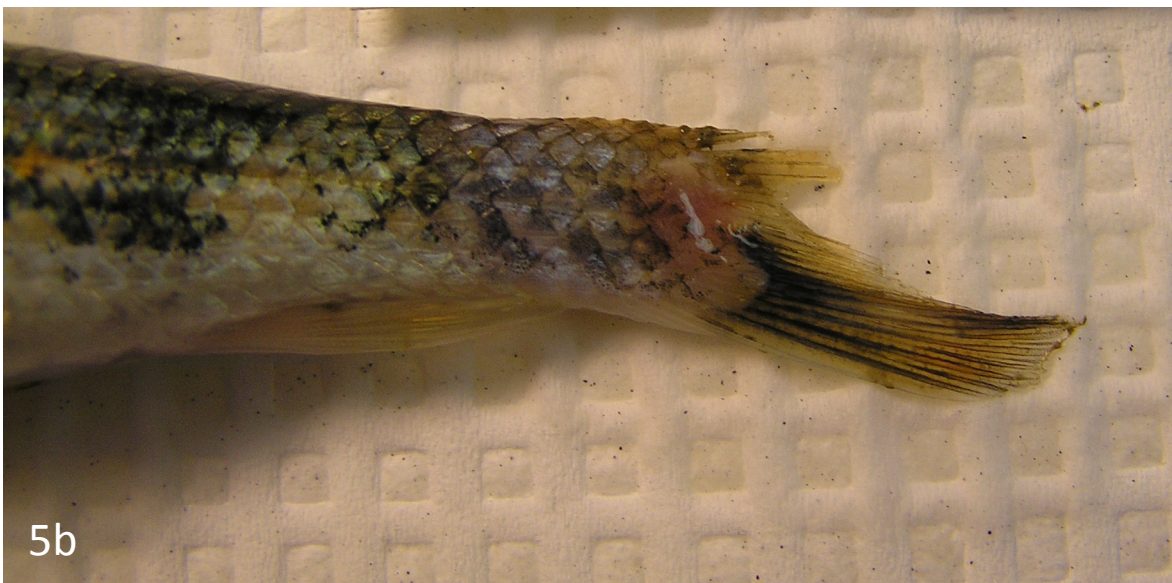
**Figure 2.** Small foci and likely early-stage lesion (see arrow) adjacent to the lateral line of a smallmouth bass (*Micropterus dolomieu*) from the Laceyville Site, Susquehanna River, PA., August 4, 2010.



**Figure 3.** Large area of extensive necrosis on the side of a smallmouth bass (*Micropterus dolomieu*) from the Clemson Island Site, Susquehanna River, PA., July 19, 2010.



**Figure 4.** Severe necrotic lesion with a wide margin covering a large portion of the side of a smallmouth bass (*Micropterus dolomieu*). This fish was caught on July 13, 2010 from the Juniata River Site, Susquehanna River, PA.



**Figures 5a and 5b.** Pathology to fish collected at the Clemson Island Site, Susquehanna River, PA., July 19, 2010. Discoloration, scale loss and minor tissue necrosis are evident on the caudle peduncle on the smallmouth bass (*Micropterus dolomieu*) (5a), as well as complete erosion of part of the caudle fin on the golden shiner (*Notemigonus crysoleucas*) (5b).

INSTRUCTIONS TO AUTHORS  
(<http://www.marshall.edu/wvas/AUTHORS.HTML>)

## 1. General Policy

The publications policy of the Academy is intended to implement the goal of publication of the *Proceedings* by the Academy, namely, stimulation of research on the part of West Virginia scientists and Academy members by providing an outlet for publication of their research results. Within the limits of available resources, the Academy will attempt to maximize the number of articles it can publish, while maintaining standards by the peer review process. Where selection must be made, the sole criterion for judgment shall be the quality of the research involved. Articles of a local or regional nature, as well as those of broader scope, are encouraged. Articles will not be discriminated against because of their subject matter, as long as they satisfy the requirement of the bylaws (<http://www.marshall.edu/wvas/WELCOME.HTML>; click on the Bylaws link) that they be "...of a scientific nature" (Section VII, Article 1).

The Academy will consider papers that report the results of original research or observation. The Academy will not publish papers that have been published elsewhere. Each manuscript will be reviewed by the Publications Committee and by referees. Manuscripts longer than 15 pages of double-spaced, typed copy normally will not be accepted. Membership in the Academy is a requirement for publishing in the *Proceedings*. In the case of joint authorship, at least one author must be a member of the Academy. No author, or co-author, may submit more than two papers for any volume of the *Proceedings*. Ordinarily, papers offered for publication must have been presented at the annual meeting of the Academy but presentation is not a requirement for publication. Publication is not automatic. The *Proceedings* editors also solicit outstanding expository papers.

## 2. Abstract for Annual Meeting

A 'call-for-abstracts' announcement is mailed to each member in the fall.

The abstract will be formatted in the following manner:

JOHN SMITH, Dept. of Biological Mathematics, West Virginia University, Morgantown, WV, 26506,  
and JIM DOE, Dept. of Chemical Sociology, Marshall University, Huntington, WV 25755.

**Analysis of trigonometric cell structure in the chromosome.**

Skip one line and begin the first paragraph of text. Single-space the text. Start each new paragraph by indenting 0.25" (1/4") using a tab, not the space bar. Do not skip a line between paragraphs. Standard ab-breviations may be used. The abstract should contain a brief statement of (a) the objectives of the study, (b) the method of study used, (c) the essential results including data and statistics, (d) the conclusions, and (e) the source of support (if applicable). Figures and tables cannot be accommodated. Please check the abstract for misspellings, poor hyphenation, and poor grammar. The text of the abstract should not exceed 250 words.

### 3. Manuscripts

Manuscripts for publication should be sent to the editor, Dr. Richard Ford, 101D Hamblin Hall, Rt. 25 and Barron Drive, Box 1000, Institute, WV 25112-1000. Manuscripts must be sent electronically (email or compact disk) in Microsoft WORD to fordri@wvstateu.edu. One hardcopy should also be sent to the address above. Proofs, edited manuscripts, and all correspondence regarding papers for publication should be directed to the editor. For additional information, call (304) 766-5742.

#### a. Cover-sheet (Title and by-line)

The cover sheet for each manuscript should include the title (bold, 12-pt. New Times Roman font) of the paper followed by the names and business addresses of all authors. The corresponding author should be indicated by an asterisk and include a business phone number, fax number (if available), and e-mail address (if available)

#### b. Organization of Manuscripts

Each manuscript shall start with an abstract (no more than 250 words) that should summarize the primary results. In general, the introductory abstract will replace a summary. This abstract should be suitable for sending to international abstracting services for immediate publication in the event that the paper is accepted for publication in the *Proceedings*.

The following sequence is suggested for organizing a paper: Introduction, Materials and Methods, Results, Discussion, Acknowledgments, and Literature Cited.

The text should be double-spaced (New Times Roman 12 pt. font size), and pages should be numbered consecutively in the top right-hand corner of each page preceded by the author's last name.

Major section headings (**INTRODUCTION, METHODS**, etc.) are to be bold and all caps and subsection headings should be presented in 10-pt font size, in all caps but not bolded.

Using a tab, not the space bar, indent each paragraph 0.25" (1/4").

#### c. Grammatical Considerations

Place two spaces between the period at the end of one sentence and the first letter of the next.

Hyphenate compound modifiers and compound words. A modifier made up of an adverb (other than adverbs ending in -ly) + adjective, adjective + noun, or two nouns is a compound or unit modifier., eg., *plum-pox-resistant*, *transgenic plum*, where *plum-pox-resistant* is the compound modifier (hyphens are boldface for emphasis). Note: chemical names used as modifiers are not hyphenated except when misinterpretation is likely. Examples: 1. Iron sulfide containing bacteria is commonly found ... ; 2. Iron sulfide-containing bacteria are ... (In example 1., a sample of iron sulfide that contains bacteria within it is the subject; in example 2., the bacteria contain iron sulfide and *bacteria* is the subject. Include a comma after each member in a series of words that form a list in a sentence, form a series of



modifiers modifying the same item, or for a series of phrases, as this sentence itself exemplifies, e.g., ... *dogs, horses, antelope, and trout*... A different example exemplifies an important exception: when an adjective or noun acting as an adjective is conceptually very closely related to the immediately following noun, as *big* in **big apple**, it is not considered part of the series of modifiers modifying the noun. Thus in ...*moldy, green, foul-tasting big apple* ... commas follow all of the modifiers prior to *foul-tasting*, but because *big* is closely associated with *apple*, it is not in the series; hence *foul-tasting* is the last modifier in the series (it could have been preceded by *and*).

Latin epithets used in scientific names for animals and plants follow a different set of rules than English names, even “official” English names. The guideline for English names is based on the rule “only proper nouns are capitalized in sentences”, e.g., *coastal plain oak, raspberry horntail sawfly* would not be capitalized in a sentence. Capitalize the first letter of the first word in a sentence and capitalize the first letter for each major term in titles, figure captions, and table headings. Note: the symbol *pH* always has a lowercase *p* and uppercase *H*; it should not be the first “word” in a sentence, caption, or title if things can be conveniently rearranged.

Spell out numbers “one” through “nine”; use numerals for numbers higher than nine. As with *pH*, avoid beginning sentences, captions, and titles with a numeral.

There exist hyphens, en-dashes, and em-dashes, and each has a use. One should distinguish especially between the hyphen (the shortest of these marks) and the en-dash (the intermediate in length of the three). The en-dash should be used in two-word concepts (e.g., *nickel–metal hydride battery*) and spans of time (e.g., *for the period January–June*), among other situations. In “Word” for PCs, the en- and em-dashes are available in the “Special Characters” tab of the “Symbol” sub-menu, which is under the “Insert” menu. In Macintosh computers, the en-dash is also available directly when the “alt/option” key is held down while striking the hyphen key.

For other grammatical considerations please consult a good scientific writing reference, such as the *Scientific Style and Format: The CSE Manual for Authors, Editors, and Publishers* by Council of Science Educators Style Manual Committee.

#### 4. Figure, Illustrations, and Table Preparation

Each table or figure should be supplied with a legend sufficiently complete to make the table or figure intelligible without reference to the text. Footnotes may be used in connection with tables and figures where necessary. Footnotes should be avoided whenever possible in the text itself. Complicated formulas should be prepared with care in a form suitable for camera copy reproduction. Avoid such formulas in the text. Acceptable fonts include Times, Times New Roman, Arial, Courier, Helvetica, and Symbol. Table and figure format should follow those in issue **79**(2) or later.

Example Table: Table 1. Synthesis of PIT tag retention rates from American eel studies.

Study	Location of Study	Duration	Eel Length (mm)	Tag Location	Tag Retention
Thomas (2006)	Laboratory	6 months	≥ 500	Dorsal musculature	100%
Morrison and Secor (2003)	Hudson River, NY	2 months	Mean = 457	Visceral cavity	89%
Verdon and Desrochers (2003)	St. Lawrence River, NY	1998-1999	Mean = 471.7 (1998) Mean = 468.7 (1999)	Behind the head	98%
Verdon <i>et al.</i> (2003)	Richelieu River, Quebec	1997-1999	Mean = 379.7	Dorsal Musculature	93.9%

Prepare figures and illustrations to be close to the expected size within the publications, with a width of no less than 3 inches (column width) or 6.5 inches for full-page width.

All illustrations and photographs will be published in black and white or grayscale. Use shaded fills for shapes and graphs. For figures with bars, shading, diagonal, and horizontal lines are allowable. Each bar fill-type should be clearly distinct. All drawn lines must be greater than 0.25 pts (0.1 mm) thick. All figures should have a white chart area. See *WVAS Proceedings 79(2)* or later for example formatting.

The recommended file format and resolution for various types of line drawing and photos are:

- Black and white line art, use 1000 dpi minimum resolution
- Half tone and grayscale – use minimum resolution of 600 dpi
- Images and photos need to be in grayscale with a minimum resolution of 600 dpi

All illustrations should be submitted electronically as a separate file for each figure. Acceptable file formats are TIF, PDF, Microsoft PPT, DOC, or XLS. No other formats are accepted at this time.

**Please note: Illustrations, graphs, and photos that do not comply with the recommended format will be returned to the author for correction. The manuscript will not be considered for review until it is resubmitted with the required corrections. Figures and tables covering more than one page should have the figure or table number repeated at the top of each of the other pages followed by the word “continued” within parentheses. Data, legends, and other identifiers that appear within a figure or table need to be large enough in the published version to be easily read.**

## 5. Literature Cited

References shall be collected at the end of the manuscript as “Literature Cited” and must be cited in the text.

- Citations within text:

References should be cited by author and date within the text. Separate multiple citations with a semicolon.

- Example citations within text:

Single author: (Dare 2003)

Two authors: (Buzby and Deegan 1999)

Multiple authors: (Feldheim *et al.* 2002)

Multiple citations: (Buzby and Deegan 1999; Feldheim *et al.* 2002)

- Citations at the end of paper:

The title of the papers cited and the inclusive page numbers must be given.

The article title should be italicized and the journal name should be in normal font.

Bold the volume number, italicize the issue, and present page numbers in normal font.

End each citation with a period.

Citations should be formatted with hanging indentation of 0.5”.

Do not skip a line between citations.

- Example journal citations:

Buzby, K. and L. Deegan. 1999. *Retention of anchor and passive integrated transponder tags by arctic grayling*. N. Am. J. Fish. Manage. **19**(4): 1147-1150.

Dare, M.R. 2003. *Mortality and long-term retention of passive integrated transponder tags by spring Chinook salmon*. N. Am. J. Fish. Manage. **23**: 1015-1019.

Feldheim, K.A., S.H. Gruber, J.R.C. de Marignac, and M.V. Ashley. 2002. *Genetic tagging to determine passive integrated transponder tag loss in lemon sharks*. J. Fish Biol. **61**: 1309-1313.

Example book citation:

Stacey, M. and S. A. Barker. 1960. *Polysaccharides of microorganisms*. Oxford Univ. Press. London. 228 pp.

Freemark, K. and B. Collins. 1992. *Landscape ecology of birds in temperate forest fragments* in J. M. Hagan, III and D. W. Johnston (eds.), *Ecology and Conservation of Neotropical Migrant Landbirds*, pp. 443-454. Smithsonian Institution. Washington, D.C.

## **6. Submission of Revised Manuscripts**

All manuscripts accepted by the peer reviewers that need to be revised must be done according to instructions and submitted to the editor either by e-mail or on a compact disk.

## **7. Proof**

If galley proofs are sent to authors for corrections they should be made on margins of the proof. Proof-reader's marks may be found in dictionaries and in style manuals (e.g., "Style Manual for Biological Journals"). Changes in text after the manuscript is in galley proof are quite expensive and in general are not permitted. Galley proofs must be corrected and returned promptly (within ten days).

## **8. Reprints**

A reprint order blank will be sent with the galley proofs. This should be returned with the corrected proof.

## **9. Cost of Publication**

Authors will be billed by the Academy for pages in excess of the maximum allowed (15 pages—see item 1). The cost of figures that require half-tone screens, such as photographs, will also be billed to the authors. Currently, a page charge of \$15.00 per page is in effect, and the author will be sent a pro forma invoice to see if payment can be secured from the author's institution, company, research grant, etc. Failure to honor page charges will not prevent publication of a paper, but will greatly assist the publication program of the Academy.